ÉTUDE DES VARIATIONS NATURELLES DE LA PROTEINE VEGETALE ARGONAUTE 2 ET DE LA RESISTANCE VIRALE

par

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A STUDY OF THE NATURAL VARIATIONS IN THE PLANT ARGONAUTE 2 PROTEIN AND VIRAL RESISTANCE

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SOMMAIRE

La neutralisation de l'ARN est un mécanisme majeur de défense antivirale constitutive chez les plantes. Les protéines Argonaute (AGO) et les protéines de type dicer (DCL) ont été identifiées comme étant les principaux acteurs de la voie de silençage de l'ARN. En ciblant l'ARN double brin issu d'une infection virale, les plantes peuvent utiliser le silençage de l'ARN en tant qu'outil antiviral efficace. Le génome de la plante modèle, Arabidopsis thaliana, code pour dix protéines AGO et quatre protéines DCL. Des études antérieures ont révélé que, même si toutes les AGO possèdent une capacité intrinsèque de cibler l'ARN viral en fonction du virus en question (Brosseau et Moffett, 2015), les AGO 1 et 2 sont les AGO les plus étudiées pour la défense contre les virus à ARN.

Les travaux effectués au cours de mes études ont porté sur la variabilité génétique présente dans le gène AGO2. Afin de déterminer le (s) rôle (s) de la variation génétique dans le gène AGO2 au cours d'infections virales, les polymorphismes présents dans le gène AGO2 dans des accessions d'Arabidopsis naturelles ont été évalués. Ces accessions étaient infectées par un *Potexvirus*, un *Potyvirus* et un *Cucumovirus*. Sur la base de travaux antérieurs dans notre laboratoire (Brosseau et al., 2019), nous avons classé ces accessions en tant qu'accessions de type Col-0 AGO2 ou en tant que accessions de type C24 AGO2. Les résultats de cette étude montrent que l'allèle AGO2 de type Col-0 est nécessaire à la défense contre potex et poty, alors que l'allèle AGO2 de type C24 ne confère pas de résistance aux infections virales. Bien que l'allèle C24 AGO2 ne soit pas important pour la résistance à l'infection virale, nous montrons que cet allèle est important pour les efforts de reproduction en l'absence de virus. Cela a révélé un compromis entre reproduction et défense. Étant donné que les deux allèles AGO2 sont présents à une fréquence élevée, l'allèle C24-AGO2, qui confère une sensibilité aux virus, a été retenu pour son avantage en matière de reproduction. Un résultat qui n'a jamais été rapporté pour un gène AGO.

Les variations interspécifiques de la protéine AGO2 ont également été explorées. En développant des plantes transgéniques de *Nicotiana benthamiana* et de tomates possédant une AGO2 d'Arabidopsis, on a évalué l'accumulation de PVX. Dans ces plantes transgéniques, AtAGO2 a

permis de limiter le mouvement et l'accumulation de PVX dans les feuilles systémiques. Nous rapportons que les plantes transgéniques étaient plus tolérantes à l'infection à PVX. En conclusion, les travaux présentés dans cette thèse étudient les variations naturelles présentes dans et leur importance pour la résistance aux virus. En outre, en utilisant une approche de résistance sans hôte, l'ingénierie de plantes résistantes aux virus était réalisable.

Mots-clés: ARN inhibant, plante, Argonaute, défense antivirale, variation naturelle, transgénis, tolérance virale.

ABSTRACT

RNA silencing is a major mechanism of constitutive antiviral defense in plants. Argonaute proteins (AGO) and dicer-like proteins (DCL) have been identified to be the major players in the RNA silencing pathway. By targeting double-stranded RNA derived from infection from viruses, plants are able to use RNA silencing as an efficient antiviral tool. The genome of the model plant, *Arabidopsis thaliana* (*A. thaliana*) encodes ten AGO proteins and four DCL proteins. Previous studies have revealed that although all AGOs possess an intrinsic ability to target viral RNA depending on the virus in question (Brosseau and Moffett, 2015), AGOs 1 and 2, are the most studied AGOs for defense against RNA viruses.

Work done during my studies, focused on the genetic variability present within the *AGO2* gene. In order to determine the role(s) of genetic variation within the AGO2 gene during virus infections, the polymorphisms present in the *AGO2* gene in natural *A. thaliana* accessions was assessed. These accessions were infected with a *Potexvirus*, a *Potyvirus* and a *Cucumovirus*. Based on previous work in our lab (Brosseau et al., 2019), we classified these accessions as Col-0-like *AGO2* accessions or C24-like *AGO2* accessions. Results from this study show that the Col-0-like *AGO2* allele is necessary for defense against certain virus whereas the C24-like *AGO2* allele does not confer resistance to virus infections. Although the C24 *AGO2* allele is not important for resistance against virus infection, we show that the said allele is important for reproductive efforts in the absence of viruses. This revealed a trade-off between reproduction and defense. Since both *AGO2* alleles are present at high frequency in nature, the C24-*AGO2* allele, which confers susceptibility to viruses, has been retained due to its conferring a reproductive advantage. A result that has never been reported for an *AGO* gene.

The interspecies variations in the AGO2 protein was also explored. By developing transgenic *N. bethamiana* and tomato plants that express an *A. thaliana* AGO2 (AtAGO2) protein, the accumulation of PVX was monitored. In these transgenic plants, AtAGO2 helped to curtail the movement and accumulation of PVX in upper non-inoculated leaves. We report that the transgenic plants were more tolerant to PVX infection. In conclusion, work presented in this thesis

investigates the natural variations present in and how these variations are important for virus resistance. Also, by utilizing a non-host resistance approach, the engineering of viral resistant plants was achievable.

Keyword: RNA silencing, plant, Argonaute, antiviral defense, natural variation, transgenics, viral tolerance.

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LISTS OF ABBREVIATIONS

Abbreviations	Full name
+ssRNA	positive-sense single-strand RNA
AGO	ARGONAUTE(S)
At	*Arabidopsis thaliana*
BaMV	Bamboo mosaic Virus
BMV	Brome mosaic virus
bp	base pair
CaLCuV	*Cabbage leaf curl virus*
CaMV	*Cauliflower mosaic virus*
cDNA	complementary DNA
CMV	*Cucumber mosaic virus*
CDS	Coding DNA sequence
CP	Coat protein
CRP	Cysteine-rich protein
CymRSV	*Cymbidium ring spot tombusvirus*
DCL	Dicer-like
DNA	Deoxyribonucleic acid
dpi	day post-inoculation/ days post-infection
DRB	double-stranded RNA binding protein
dsRNA	double-stranded RNA
ETI	Effector triggered immunity
EV	Empty vector
GFP	Green fluorescence protein
HA	Hemagglutinin
HEN1	Hua Enhancer
hcRNA	Heterochromatic siRNA
HR	Hypersensitive Response

HRP	Horseradish peroxidase
MAMPs	Microbe-associated molecular patterns
$MgCl_2$	Magnesium Chloride
MID	Middle
miRNA	microRNA
MP	Movement protein
Nb	*Nicotiana benthamiana*
Nt	*Nicotiana tabacum*
nt	Nucleotide
NB-LRR	*Nucleotide-binding, leucine-rich repeats*
OD	Optical density
Prom	Promoter
PAGE	*Polyacrylamide gel electrophoresis*
PCR	Polymerase chain reaction
PlAMV	Plantago asiatica mosaic virus
PTGS	Post-transcriptional gene silencing
PTI	PAMP-triggered immunity
PVX	Potato virus X
RdDM	RNA-dependent DNA Methylation
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulfate
SGS3	Suppressor of gene silencing 3
sRNA	Small RNA
siRNA	Small interfering RNA
TBSV	Tomato bushy stunt virus
TCV	Turnip crinkle virus
T-DNA	Transfer DNA
TGB	Triple gene block
TGS	Transcriptional gene silencing
ToRSV	Tomato ringspot virus

TuMV	Turnip mosaic virus
UV	ultraviolet
VRC	Viral replication complex
VSR	Viral suppressor of RNA silencing
WT	Wild type

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CHAPTER 1[1]

GENERAL INTRODUCTION

1.1 Plant Defense Responses.

In nature, plants, like animals are attacked by microorganisms in their environment. Due to pathogen infection, an estimated 30% of crop production is lost both before and after harvesting (Jones et al., 2016). Therefore, protection against diseases and pests are vital for sustainable agriculture (Jones et al., 2016). These pathogens include bacteria, viruses, nematodes, insects and fungi. However, to deal with these challenges, plants have evolved a number of ways to combat these biotic stresses (Chisholm et al., 2006; Dempsey and Klessig, 2012; Jones et al., 2016; Wei et al., 2015). Plant defense against pathogens is based on a multi-layer immune system that includes pathogen (or microbial)-associated molecular pattern (PAMP)-triggered immunity (PTI), effector-triggered immunity (ETI), as well as a type of whole plant immunity known as systemic acquired resistance (SAR) (Chisholm et al., 2006; Dempsey and Klessig, 2012; Jones et al., 2016; Wei et al., 2015).

The immune system of plants plays a major role in inhibiting the growth of pathogens, therefore, an important aspect of the plant immune system is that each plant cell has the ability to recognize pathogens (Jones et al., 2016). In many cases, plant immune responses are initiated by the recognition of the invasion of pathogens through immune receptors (Boutrot and Zipfel, 2017). A first level of recognition is performed by a class of host cell surface transmembrane proteins called pattern recognition receptors (PRRs) (Jones and Dangl, 2006; Pieterse et al., 2012). PRRs recognize specific conserved structures on pathogens, often referred to as pathogen (or microbe) associated molecular patterns (PAMPs or MAMPS), which includes proteins (such as flagellum

[1]Some information in this chapter has been readapted and taken from: An original review article to be submitted to the *Journal of Experimental Botany*. Guilherme Silva Martins*, Ayooluwa Adurogbangba* and Peter Moffett (2019). What does it take to be antiviral? An Argonaute Centered Perspective in Plant Antiviral Defense. (*both authors contributed equally to the work).

and elongation factor Tu), carbohydrates (such as fungal chitin), lipopolysaccharides (Felix et al., 1999; Kunze et al., 2004; Albert, 2013) and other molecules that are essential for microbial survival. Once a PAMP is recognized, a cascade of defense responses is triggered (Nicaise et al., 2015), which leads to the prevention of further pathogen ingression (Cook et al., 2015; Nicaise et al., 2015). Plants show similar responses upon the recognition of diverse PAMPs. There is an activation of PRRs, which results in intracellular signaling and the modulation of gene expression within the host, thereby leading to defense responses that restrict microbial proliferation in the host. Also, break-down products of host molecules caused by wounding or infection, known as damage-associated molecular patterns (DAMPs), may induce PTI-like responses following interactions with host PRRs (Tang et al., 2007; Yamaguchi et al., 2010).

Pathogens deploy effectors that contribute to the virulence of the pathogen - proteins encoded by pathogens that interfere with PTI by preventing the detection of PAMPs or by suppressing downstream signaling, thereby enhancing pathogen proliferation within the host (Nicaise et al., 2015). ETI is activated upon the specific recognition of effector proteins by specific nucleotide-binding and leucine-rich-repeat proteins (NLR) proteins, which leads to an induction of a robust immune response. NLR proteins encode either an N-terminal coiled-coil (CC) or Toll-interleukin receptor (TIR) domain, a central nucleotide-binding (NB) and a C-terminal leucine-rich repeat (LRR) domain (McHale et al., 2006). How NLRs induce ETI is poorly understood (Lai and Eulgem, 2018). ETI often results in the development of a hypersensitive response (HR), a form of programmed cell death which, together with other defense reactions provide protection against pathogens (Cook et al., 2015; Jones et al., 2016; Stael et al., 2015). However, HR is not observed in all cases of ETI (Coll et al., 2011).

Plants encode resistant genes (*R* genes) that confer disease resistance to pathogens (Cook et al., 2015; Garner et al., 2016). The majority of *R* genes encode NLR proteins, which recognize, directly or indirectly, pathogen effectors. Historically, effectors recognized by *R* genes were called avirulence (*Avr*) factors as they make pathogens avirulent on plants carrying the *R* genes, whereas they contribute to virulence on plants without the cognate *R* gene (Liang Wu, Huan Chen, 2014). One could look at this model in a co-evolutionary context where natural selection drives pathogens to diversify their effectors to overcome *R* genes and plants will benefit from the advantage of

having expanded and diversified *R* gene repertoires, leading to the so–called zigzag model (Jones and Dangl, 2006). In the zig-zag model, there are four phases. In the first phase, PAMPs/MAMPs are recognized by PRRs, leading to PTI that alters further pathogen colonization (Figure 1.1). However, in phase 2, successful pathogens deploy effectors, which contributes to the virulence of pathogen. These effectors interfere with PTI, leading to effector-triggered susceptibility (ETS). In phase 3, an effector is recognized by an NLR protein, resulting to ETI and an HR is often observed at the infection site. Lastly, in phase 4, the pathogen avoids ETI by diversifying the recognized effector gene or by producing additional effectors (Jones and Dangl 2006) (Figure 1.1). Therefore, for successful invasion, pathogens need to suppress the plant innate immunity to cause diseases.

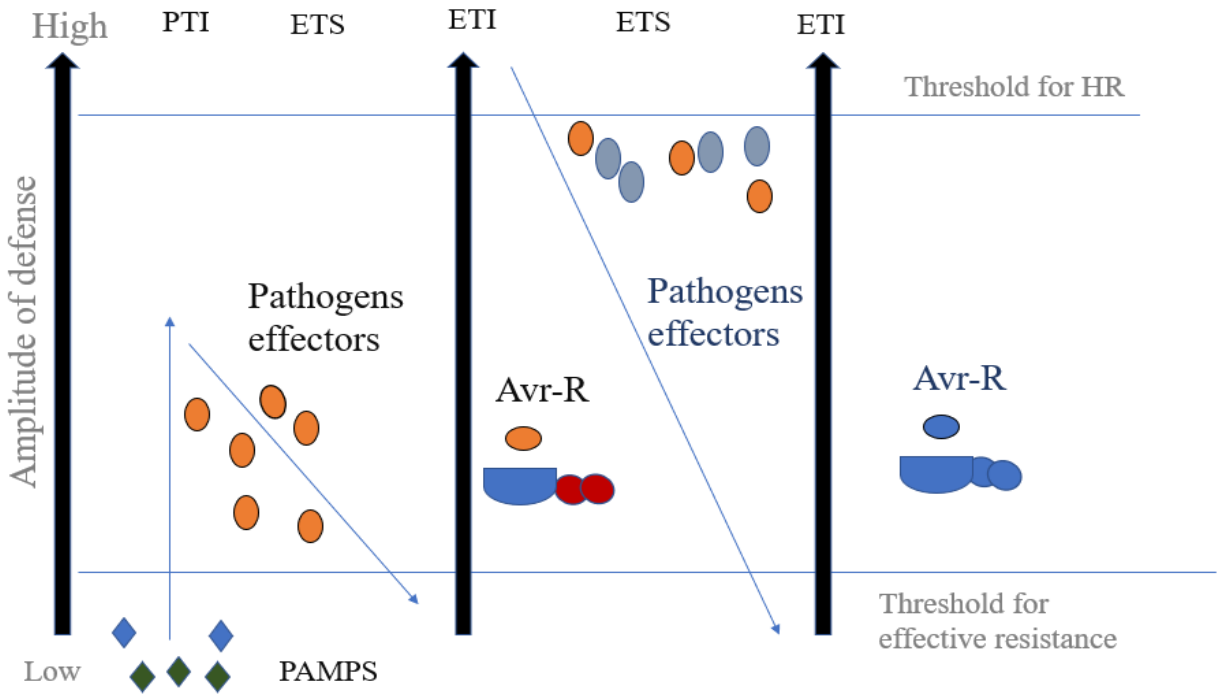

Figure 1.1: The zig-zag model of Plant innate immunity with all the four phases.
During phase 1, through PRR, the plant detects MAMPs/PAMPs (green and blue diamonds) and triggers PAMP-triggered immunity (PTI). In the second phase, effectors are delivered during successful invasion by the pathogens, leading to effector triggered susceptibility (ETS). For phase 3, there is a recognition of an effector (depicted in brown) by an NLR protein (blue and red), resulting in effector-triggered immunity (ETI). Phase 4 shows a situation wherein a pathogen has

lost the brown effector and maybe gained new effectors (blue). This leads to the avoidance of ETI but ETI can eventually be restored as the plant evolves new NLR alleles. Adapted from (Jones and Dangl, 2006).

1.1.2 Introduction to RNA Silencing

In plants, an additional defense mechanism, largely specific to antiviral defenses involves the RNA silencing pathway, which is based on 20- to 30- nucleotide RNAs. RNA silencing is a series of related processes in which small RNAs target nucleic acids for regulation in a sequence-specific manner (Fang and Qi, 2016). Small RNAs (sRNA) are key players in RNA silencing pathway and sRNAs are involved in plant development, the reprogramming of the genome and reproduction (Borges and Martienssen 2015). RNA silencing targets double stranded RNA (dsRNA) from endogenous and exogenous invading nucleic acids such as viruses and transposable elements (TE) (Borges and Martienssen 2015). The major classes of sRNAs are microRNAs (miRNAs) and small interfering RNAs (siRNAs) (Fang and Qi, 2016; Kim, 2005; Shamandi et al., 2015). miRNAs are involved in post transcriptional gene silencing (PTGS) through the cleavage of transcripts or the repression of translation and miRNAs also trigger the production of secondary siRNA transcripts (Fang and Qi, 2016; Kim, 2005). miRNAs are derived from precursor RNAs with partially double-stranded regions (Borges and Martienssen, 2015; Jouannet et al., 2012; Moreno et al., 2013). The miRNA pathway is initiated with the transcription of *MIRNA* genes by RNA polymerase II, forming a 5'capped and poly-A tailed pri-miRNA (Axtell, 2013). These miRNAs negatively regulate gene expression by pairing with the appropriate mRNA bases as part of a RISC complex, subsequently resulting in either RNA cleavage or an inhibition of protein translation (Axtell, 2013). RNA silencing can also be triggered by exogenous or endogenous dsRNA precursors, including dsRNA viral replication intermediates and local self-complementary double-stranded regions of viral genomes (Borges and Martienssen, 2015; Zhang et al., 2015), making RNA silencing a first line of antiviral immunity in plants (Ding and Voinnet, 2007; Dunoyer et al., 2006).

The mechanism of RNA silencing begins with long double-stranded RNA duplexes (Fang and Qi, 2016; Shamandi et al., 2015), and these duplexes are recognized by members of a protein family called dicer-like (DCL), proteins (Fang and Qi, 2016; Shamandi et al., 2015). These DCL proteins

cleave the dsRNA into sRNAs that are usually 21 to 24 nucleotides in length (Fang and Qi, 2016; Shamandi et al., 2015). Upon generation by DCLs, sRNAs are bound by a second family of endoribonucleases, the Argonaute (AGO) proteins, (Wang et al., 2019) which form the core of what are known as RNA-induced silencing complexes (RISC). AGO proteins allow RISC complexes to be guided by the sRNA to target RNA or DNA molecules through base-pair complementarity (Figure 1.3). Following sRNA-target binding, AGO proteins either cleave targeted RNA or repress its translation. Alternatively, in the case of DNA targeting, AGO proteins modify chromatin through recruitment of the RdDM machinery (Fang and Qi, 2016; Zhang et al., 2015). Although RNA silencing components are involved in a plethora of gene regulatory mechanisms, for this thesis, emphasis will be placed on the role of AGO proteins in defense against viruses.

Finally, in plants, RNA silencing also functions in association with DNA methylation and suppression of transcription (Carbonell and Carrington, 2015). This RNA silencing pathway occurs at the chromatin level and has been found to protect plant genomes against damage caused by transposons (Lario et al., 2013; Paulo et al., 2017) (see below for more details). The aforementioned silencing pathways are present in plants, however other organisms may have lost one or more of these pathways (Bologna and Voinnet, 2014; Borges and Martienssen, 2015; Moreno et al., 2013). For this thesis, emphasis will be placed on the cytoplasmic siRNA pathway due to the nature of the research projects to be discussed.

1.2.1 Origin and biogenesis of siRNAs and the role of RDRs.

Research has shown that siRNAs protect the genome in various ways, such as suppressing viruses that try to invade the cells, silencing transposable elements and repetitive elements in the genome, as well as silencing of aberrant transcripts and genes in the genome (Yu et al., 2016; Zhang et al., 2015). There are a number of molecular mechanisms involved in RNA silencing. Several of these pathways are explained in detail below. In addition to the sources of dsRNA described above, dsRNA may arise from the hybridization of sense and antisense mRNA transcripts (Di Serio et al., 2001). dsRNA molecules may also be synthesized by RNA-dependent RNA-polymerases (RdRPs/RDR) with or without initial priming. Priming is the synthesis of a short strand of RNA,

initiating polymerase-catalyzed synthesis of long dsRNA. RDRs are defined by the presence of a conserved RNA-dependent RNA polymerase catalytic domain (Willmann et al., 2011). There are 3 major clades of eukaryotic RDRs: RDRα, RDRβ and RDRγ. RDRα is reported to be found in fungi, plants and in animals, while RDRγ is only found in plants and RDRα is present in only animals and fungi (Wassenegger and Krczal, 2006). In *A. thaliana*, there are 3 types of RDRα: RDR1, RDR2, RDR6 and three types of RDRγ: RDR3, RDR4 and RDR5. While the RDRγ clade remains functionally uncharacterized in plants, its significance is based on its presence in many fungi and its involvement in transcriptional gene silencing (TGS) in the fission yeast *Schizosaccharomyces pombe* (Wassenegger and Krczal, 2006; Yoshikawa, 2013). One or more of the 6 RDR paralogs in plants are involved in strengthening silencing responses by the production of dsRNA via viral templates (Molnar et al., 2010; Qi et al., 2009). Initially, RDRs were studied due to their roles in antiviral plant defense and transgene silencing (Wang and Metzlaff, 2005), however, RDRs also have other molecular functions that include control of chromatin structure and the regulation of cellular gene expression (Wassenegger and Krczal, 2006). RDR1 is involved mostly in the amplification of exogenous, virus-induced small RNAs, making it a part of the plant antiviral RNA silencing system, in conjunction with DCL2 and DCL4 (Xiaoming Zhang et al., 2012). RDR2 plays a role in DNA methylation by converting the ssRNA produced by polymerase IV into dsRNA. RDR6 functions in both endogenous trans-acting siRNA biogenesis as well as antiviral silencing (Yoshikawa et al., 2005; Curaba et al., 2008; Garcia-Ruiz et al., 2010).

For RNA viruses to be replicated, virus-encoded RDRs are required (Pogany and Nagy, 2015, 2012). During an infection, RDRs act in concert with other viral and host cellular factors that play a role in RNA synthesis, RNA elongation, and other functions (Pogany and Nagy, 2015). In some viruses, RDR proteins are reported to be inactive in the cytoplasm to prevent the formation of viral dsRNA that could trigger RNA silencing (Pogany and Nagy, 2012). Therefore, the activation of viral RDRs is an important step for viral infection (Pogany and Nagy, 2012). In the initial stages of RNA virus infection, the plus stranded RNA is released from the virion and produces the viral RDR using the host translation machinery. The viral RDR then produces the minus strand using the plus strand RNA (Newburn and White, 2015; Verchot-Lubicz et al., 2010).

As a mechanism of amplification of antiviral response, plants generate secondary viral siRNA (vsi-RNA) that can spread systemically to neighboring cells via plasmodesmata (PD) and the phloem. The amplification of the silencing signal is mainly dependent on host RDR1/6 and suppressor of gene silencing 3 (SGS3) proteins. These steps start with the *de novo* synthesis of viral dsRNA with or without initial priming from "aberrant" viral RNA cleavage products. One strand of the vsiRNA can act like a primer for the dsRNA production by RDRs (Molnar et al., 2010; Verlaan et al., 2013; Wang et al., 2010). This secondary dsRNA is processed by DCL proteins to form secondary vsiRNA, thereby amplifying the antiviral RNA-silencing.

1.3 Dicer and Dicer-like proteins.

The enzymes responsible for producing siRNA and miRNA from dsRNA are Ribonuclease (RNase) III-like enzymes belonging to the Dicer family (Vickers et al., 2003).
A. thaliana encodes four specialized Dicer-like (DCL) proteins named DCL1, 2, 3, and 4 (Bellaoui et al., 2003; Vickers et al., 2003). While DCL1 processes fold-back precursors to generate miRNAs, (Bellaoui et al., 2003; Liu et al., 2009; Vickers et al., 2003) plant siRNAs are processed primarily by DCL2, DCL3, and DCL4 (Liu et al., 2009). These siRNAs are known as heterochromatin siRNAs (hetsiRNAs) (Xiaoming Zhang et al., 2012). Of all these small RNAs, the 24-nucleotide hetsiRNAs are the most abundant, as they play a role in transcriptional silencing of repetitive elements within the genome with the aid of RNA-directed DNA methylation (RdDM). The role of each DCL is well established and rather specific, however, redundancy between DCL functions has been proposed (Katsarou et al., 2016).

In *A. thaliana*, it has been shown that DCL1 is the only dicer protein that produces 21-nt miRNAs (Kurihara and Watanabe, 2004; Reinhart et al., 2002) and null mutants of *dcl1* alleles are embryonic lethal (Blevins et al., 2006; Curtin et al., 2016; Tsuzuki et al., 2014.). DCL2, together with RDR6 and SGS3, has been shown to be involved in the cleavage of antisense transcripts and in the synthesis of 22-nt natural antisense- small interfering RNA (nat-siRNA) (Bellaoui et al., 2003; Liu et al., 2009; Vickers et al., 2003). DCL2 processes dsRNAs to produce 22-nt vsiRNA to be loaded into AGO proteins (Pumplin and Voinnet, 2013). DCL2 can function in antiviral defense in the absence of DCL4 and may play a redundant role in some respects (Xiaoming Zhang

et al., 2012) and 22-nt and 21-nt siRNAs are both required for optimal resistance against viral invasion (Gonzalez-Gaitan et al., 2004; Parent et al., 2015; Wang et al., 2011). The DCL4 protein is the major producer of 21-nt antiviral siRNA and endogenous siRNA such as tasiRNA and phasiRNA (Bouché et al., 2006; Qu et al., 2008; Yoshikawa, 2013). Indeed, studies have shown that in *A. thaliana*, maximal viral replication is achieved upon the mutation of both *dcl2* and *dcl4* genes (Gonzalez-Gaitan et al., 2004; Oa et al., 2010; Jaubert et al., 2011; Andika et al., 2015; Parent et al., 2015).

For certain viruses, such as cucumber mosaic virus (CMV) (Bouché et al., 2006; Fusaro et al., 2006), turnip crinkle virus (TCV) (Deleris et al., 2006) and cabbage leaf curl virus (CaLCuV), the above observation is valid (Blevins et al., 2006). However, some studies have shown situations where DCL2 and DCL4 appear to function differently. These studies include work done by (Mlotshwa et al., 2008), where DCL2 was identified as the protein responsible for secondary transitive siRNA synthesis, whereas DCL4 was shown to be involved in the production of primary siRNAs. Additional studies have also supported the above findings, demonstrating that DCL2-dependent 22-nt siRNAs do not contribute to CMV resistance (Wang et al., 2011). The inactivation of DCL4, induces a high level of viral replication, indicating that DCL4 is essential for intracellular antiviral silencing (Song and Rossi, 2017). While DCL2 can produce abundant 22-nt viral siRNAs in the absence of DCL4, these siRNAs are less efficient in mediating antiviral defense (Andika et al., 2015; Wang et al., 2011). Therefore, based on the above, it can be concluded that there could be a specialization of function of both DCL2 and DCL4 proteins.

DCL3 is reported to be important for defense against DNA viruses through DNA methylation, although the silencing suppressors of some RNA viruses interfere with DCL3 (Csorba et al., 2015). DCL3 produces 24-nt siRNAs derived from transposons and DNA repetitive elements (Daxinger et al., 2009; Priya Raja et al., 2014). These 24-nt siRNAs play an essential role in transcriptional gene silencing (TGS) by directing RdDM for the repression of transposon and DNA repeats in a TGS process (see below). Suppression of the expression of *DCL3* is linked to the enhancement of systemic PTGS (Chen et al., 2018).

1.4 Other players in the RNA silencing.

Modifications are made to sRNAs upon processing. These modifications include 2'-O-methylation, 3'-uridylation or 2'-adenylation, and adenosine deamination (Jamous et al., 2011; Zust et al., 2011). In plants, sRNAs are 2'-O-methylated at the 3'-terminal by HUA ENHANCER 1 (HEN1) to prevent uridylation (a signal for degradation) by the enzyme nucleotidyl transferase HEN1 SUPPRESSOR 1 (HENSO1) (Jamous et al., 2011; Zust et al., 2011).

dsRNA-BINDING (DRB) proteins are involved in the biogenesis of miRNA or ta-siRNA in plants (Montavon et al., 2017). Two DRBs, Hyponastic Leaves 1 (HYL1) and DRB4, have been found to be required for the proper function of DCL1 and DCL4, respectively (Montavon et al., 2017). DRB4 has 2 dsRNA binding motifs (dsRBD1 and 2) in its N-terminus (Montavon et al., 2017). The interaction of DRB4 and DCL4 leads to the generation of 21-nt siRNAs including ta-siRNa, DCL4-dependent miRNAs or vsiRNAs from either endogenous or exogenous dsRNAs (Montavon et al., 2017). In addition, DRB4 has been found to be targeted by the suppressor of silencing encoded by the DNA virus, cauliflower mosaic virus (CaMV) (Qu et al., 2008). However, studies have shown that DRB4 may not be directly involved in siRNA production but it might be involved in the stabilization of 21-nt viral siRNA. This is evidenced by the finding that there is a significant decrease in 21-nt siRNAs in mutant plants despite only a slight increase in viral RNA levels in *drb4* mutants (Montavon et al., 2017; F. Qu et al., 2008). DRB3 has also been shown to interact with DCL3 for defense against DNA viruses (Raja et al., 2014).

SGS3 is an RNA binding protein that binds RNA to prevent them from degradation before its conversion to dsRNA by RDR proteins (Okano et al., 2014). Previous studies have shown that AtSGS3 binds to, and stabilizes, RNA templates during the initiation of RDR6-mediated dsRNA synthesis (Li et al., 2017). SGS3, in cooperation with RDR6, has also been reported to have antiviral defense activities against DNA viruses (Li et al., 2017). In addition, *A. thaliana* SGS3 and RDR6 both co-localize in cytoplasmic granules called SGS3/RDR6-bodies (Li et al., 2017). Indeed, in *A. thaliana*, a lack of SGS3 increases susceptibility to some viruses, such as CMV, but not others, such as turnip mosaic virus (TuMV) and turnip vein clearing virus (TVCV) (Mourrain

et al., 2000). In contrast, during potyvirus infection, the silencing of SGS3 mRNA reduces the accumulation of viral RNA, as seen with potato virus A and soybean mosaic virus (Chen et al., 2015).

1.5 RISC Complexes and ARGONAUTE Proteins.

Primitive plants have been reported to encode only a few AGO proteins (Schuck et al., 2013; Vaucheret, 2008). The model plant *A. thaliana* encodes ten AGO proteins, designated AGO1 to AGO10, of which AGOs 1, 2, 4, 5, 7 and 10 have been shown, to varying degrees, to possess antiviral activities in certain contexts (Fang and Qi, 2016). The AGO protein family has expanded during plant evolution, leading to functional diversification. The specialization of AGO proteins in different pathways and biological processes is due to their intrinsic biochemical properties, spatiotemporal expression patterns as well as the protein and sRNA partners with which they interact. To successfully carry out their functions, AGO proteins have some key biochemical properties. Firstly, AGO proteins bind sRNAs in order to facilitate base pairing with complementary target RNAs (Fátyol et al., 2016; Hauptmann et al., 2015). Secondly, they possess RNaseH-like endonuclease through which they cleave target RNA (Fátyol et al., 2016; Hauptmann et al., 2015) and facilitate strand separations of fully complementary small RNA by passenger strand cleavage (Fátyol et al., 2016; Hauptmann et al., 2015). In addition, AGO proteins can amplify silencing responses by producing cleaved RNA fragments that can serve as new substrates for RDR. Lastly, AGO proteins may serve as platforms to which silencing cofactors may bind (Poulsen et al., 2013). It is known that in plants, animals, and fungi, AGO proteins bind to proteins containing Gly-Trp (GW) dipeptides (Fátyol et al., 2016; Hauptmann et al., 2015), which are often essential cofactors in RNA silencing (El-Shami et al., 2007; Poulsen et al., 2013).

1.6 Structure of AGO proteins.

The known biochemical functions of AGO proteins are carried out in large part by 4 domains: a variable N-terminal domain, a conserved Piwi-Argonaute-Zwille (PAZ) domain, a domain in the

middle of the primary structure (MID), and a C-terminal (PIWI) domain (Mallory and Vaucheret, 2010; Poulsen et al., 2013) (Figure 1.2). The N-terminal domain is divided into 3 elements of primary structure, called the N-terminal coil, N domain, and a domain of unknown function 1785 (DU 1785), formerly known as linker 1 (Fátyol et al., 2016; Mallory and Vaucheret, 2010). sRNA binding involves the MID and PAZ domains (Poulsen et al., 2013). The PAZ domain binds to the 3' end of sRNAs (Ma et al., 2004) while the MID domain binds to the 5' end through its 5'-phosphate-binding pocket in a nucleotide-specific manner (Ma et al., 2004; Mallory and Vaucheret, 2010; Mi et al., 2008; Montgomery et al., 2008). The PAZ and MID domains are connected by a large piece of intervening sequence referred to as linker 2 (L2) (Ma et al., 2004). The PIWI domain, which possesses slicer activity, adopts an RNaseH-like fold with an Asp-Asp-His (DDH) catalytic triad (Baumberger and Baulcombe, 2005; Liu et al., 2004; Rivas et al., 2005; Song et al., 2004). The mechanisms of sRNA loading into AGO proteins occurs when siRNA duplexes are loaded into the AGO protein by the RISC-loading complex in an ATP-dependent reaction (Iwasaki et al., 2010). Once bound to the dsRNA/siRNA duplex, the AGO protein cleaves 1 of the strands, the passenger strand, an action that triggers dissociation of the latter from the complex, resulting in a mature RISC containing only the guide strand (Carthew and Sontheimer, 2009).

AGO proteins are classified into different clades based on phylogenetic and (sometimes) functional relationships: AGO 1/10, AGO 2/3/7 AGO4/6/8/9 and AGO5 (Fang and Qi, 2016; Kapoor et al., 2008; Manavella et al., 2012; Vaucheret, 2008). A study identified specific and conserved amino acid sequences (blocks) containing motifs of unknown function in AGO proteins (Rodríguez-Leal et al., 2016). This study also showed that AGO4/6/8/9 and AGO1/10 clades have the most conserved linear organizations of blocks while AGO2/3/7 and AGO5 clades have a more variable linear organization of the said blocks (Rodríguez-Leal et al., 2016). Furthermore, domains of unknown functions that show a predictable position within a conserved primary structure were identified. Domains A-1 and B-1 were identified for AGO2/3/7 clade, AGO4/6/8/9 clade is characterized by containing the A-2 and PIWI-1 domains. AGO5 clade possesses the A-3, DUF1785-3, PAZ-3, B-3 and PIWI-2 domains. Lastly, AGO2/3/7 clade showed a conserved

sequence of blocks that incudes A-4, PAZ-3, B-4, PWI-3 and DUF1785-2 domains (Rodríguez-Leal et al., 2016).

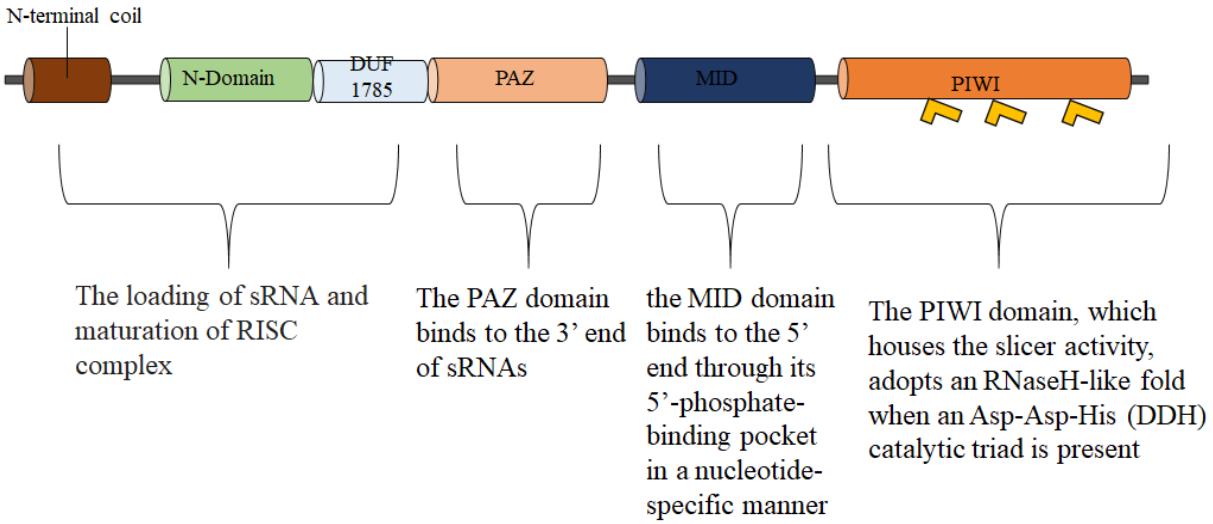

Figure 1.2. A Schematic representation of the general structure of AGO proteins. This figure displays the N-terminal domain, a domain of unknown function (DUF 1785) a conserved Piwi-Argonaute-Zwille (PAZ) domain, a domain in the middle of the primary structure (MID), and a C-terminal (PIWI) domain. Each domain is shown with an explanation of its function.

1.6.2 Classification of AGO proteins

1.6.2.1 AGO 1/10.

AGO1 is involved in the regulation of diverse miRNA genes (Vaucheret et al. 2004). AGO1 is thought to be the effector for most miRNAs and ta-siRNAs (Vaucheret et al. 2004; Baumberger & Baulcombe 2005; Qi et al. 2005; Mi et al. 2008) These miRNAs and ta-siRNAs guide AGO1 to regulate the stability and/or translation of mRNAs of genes involved in numerous developmental and physiological processes. In addition, *ago1* mutants are more susceptible to some viruses (Morel et al., 2002; Qu et al., 2008; Takeda et al., 2008; Wang et al., 2011). AGO1 protein recruits

a limited number of miRNAs and is responsible for gene regulation in specific situations. AGO1 homeostasis is regulated by miR168 through miR168-AGO1-dependent slicing of AGO1 mRNA, leading to an increase in the accumulation of miR168 in response to elevated AGO1 levels (Vaucheret et al., 2006).

A. thaliana AGO10, is the closest homolog to AGO1 in protein sequence, however, it differs to AGO1 in expression patterns and in its developmental functions (Yu et al. 2017). In *A. thaliana*, it has been shown that in shoot apical meristem, AGO10 competes with AGO1 for specific miRNAs and counters its activities through sequestration (Zhu et al., 2011). AGO10 sequesters miR165/166 thereby releasing the negative regulation they exert on the *HD-ZIP III* gene family that regulates meristem development (Zhou et al., 2015; Zhu et al., 2011). AGO10 also regulates meristem development via the mediation of translational inhibition of multiple miRNA target genes (Brodersen et al., 2008; Mallory et al., 2009). AGO10 has also been reported to have a limited role in defense against TuMV (Garcia-Ruiz et al., 2015).

1.6.2.2 AGO 2/3/7.

Proteins of the AGO2/3/7 clade show clear phylogenetic relationships but are implicated in different phenomena (Figure 1.3). The AGO2 protein of *A. thaliana* is involved in antiviral defense and is required for resistance to multiple plant viruses (Garcia-Ruiz et al., 2015; Harvey et al., 2011; Jaubert et al., 2011; Wang et al., 2011; Xiuchun Zhang et al., 2012). Biochemical experiments revealed that AGO2 loaded with synthetic vsiRNAs can target viral RNAs for cleavage, which leads to the inhibition of viral replication (Schuck et al., 2013a). AGO2, which primarily binds to 21-nt to 22-nt sRNAs, has been shown to associate with certain miRNAs (Maunoury and Vaucheret, 2011) and to regulate the production of a small number of proteins, including MEMB12, which is involved in innate immunity against bacteria (Zhang et al., 2011). AGO2 has also been implicated in DNA double-strand break repair (Wei et al., 2012) and in DNA methylation (Pontier et al., 2012), but has been studied mostly for its role in antiviral defense against multiple plant viruses (see below).

AGO3 is phylogenetically closely related to AGO2 but appears to have different biological functions (Fátyol et al., 2016). AGO3 has also been reported to recruit 24-nt sRNA for RdDM as AGO3 bound 24-nt sRNAs overlapped with those bound to AGO4; AGO4 is a key AGO in the RdDM pathway (see below) (Zhang et al., 2016). A recent study using Bamboo mosaic virus (BaMV) showed that there was an increase in expression of AGO3 upon virus infection, while an *ago3* mutant showed enhanced susceptibility to BaMV. This increase in AGO3 expression is Abscisic acid-mediated (Alazem et al., 2017).

AGO7 in *A. thaliana* is also known as ZIPPY. AGO7 binds 21-22 nt sRNAs and mediates tasiRNA biogenesis, which is important for development timing and leaf morphology (Adenot et al., 2006; Howell et al., 2007; Montgomery et al., 2008; Yoshikawa et al., 2013). AGO7 induces the secondary siRNA pathway where mi390-directed, AGO7 mediated cleavage of *TAS3* transcripts leads to the biogenesis of tasiRNA species through the action of SGS3 and RDR6 (Adenot et al., 2006; Fahlgren et al., 2006; Howell et al., 2007; Montgomery et al., 2008). TAS3 tasiRNAs are known to target several *AUXIN RESPONSE FACTOR* (*ARF*) genes that are involved in the regulation of lateral organ development and developmental timing (Adenot et al. 2006; Fahlgren 2006 et al. 2006; Hunter et al. 2006. montogomery et al. 2008). AGO7 is also known to function with DRB4, which takes part in defense against viruses (Qu et al., 2008). Upon infection with mutant TCVΔCP-lacking its viral suppressor of RNA silencing (VSR), *ago7* mutants showed higher accumulation of TCVΔCP (Qu et al., 2008). Aside the aforementioned example, AGO7 shows little involvement in defense against viruses.

1.6.2.3 AGO5.

AGO5 appears to be involved in specific developmental processes. In *A. thaliana*, *AGO5* is expressed in megaspores and the somatic cells around megaspore mother cells as well as being expressed during all stages of flower and seed formation (Kapoor et al., 2008; Schmid et al., 2005). Consistent with this, *AGO5* affects pigmentation in *Glycine max* seeds through the silencing of

chalcone synthase (Cho et al., 2017) and a semi-dominant *ago5* mutant allele leads to a defect in the initiation of mega-gametogenesis (Tucker et al., 2012). Likewise, the mutation of *MEL1*, 1 of the 5 *AGO5* clade members in rice, results in meiotic arrest, male sterility, and aberrant pollen mother cells (Nonomura et al., 2007). In addition, AGO5 may play a role in plant-microbe interactions as it appears to act in the establishment of nodules in legume-rhizobia interactions legumes (Reyero-Saavedra et al., 2017). AGO18 homologues are most closely related to the AGO5 and AGO1-containing clades and may also play important roles in development and virus defense (see below), although their activities have been less characterized than other AGO proteins (Zhang et al., 2015b).

1.6.2.4 AGO4/6/8/9, RdDM and DNA Methylation.

Although most AGO proteins are thought to function in the cytoplasm, members of the AGO4/6/8/9 clade (Xie & Yu 2015), function primarily in the nucleus. Members of this clade bind to 24-nt hc-siRNAs and target corresponding DNA sequences for RdDM through the recruitment of DNA methylation factors (Havecker et al., 2010; Zilberman et al., 2003). This results in epigenetic modifications leading to the silencing of transposons, retrotransposons and other repetitive elements, as well as controlling the expression of specific genes (Matzke et al., 2015). Proteins in the AGO4/6/8/9 clade show a high level of sequence conservation and are thought to function similarly, however they show distinct spatial-temporal expression patterns and are thought to act on different genetic elements at different times (Matzke et al., 2014; McCue et al., 2015), (Havecker et al., 2010; Mallory and Vaucheret, 2010).

RNA directed DNA methylation (RdDM), an epigenetic modification, plays a critical role in repressing transposons as well as in the regulation and maintenance of genome stability (Xie et al., 2004). Mediation of DNA methylation is performed by AGO4-bound hc-siRNAs through the RdDM pathway and catalyzed by Domains Rearranged Methyltransferase 2 (DRM2), which interacts with the Pol V complex. In this pathway, DCL3 produces 24-nt hc-siRNAs (Xie et al., 2004). The hc-siRNAs are exported into the cytoplasm, where they are loaded into AGO4, leading to an AGO4/siRNA complex. The AGO4/siRNA complexes are recruited to target loci through base-pairing with scaffold transcripts generated by RNA polymerase V (Pontier et a., 2005;

Mosher et al., 2008; Wierzbicki et al., 2009). This recruitment may be facilitated by the interaction of AGO4 with the GW/WG-rich (known as the 'AGO hook') extensions of NRPE1 (the largest subunit of Pol V) and some transcription elongation factors (Pontier et al., 2012; Pontes et al., 2006; He et al., 2009; Bies-Etheve et al., 2009). The AGO4/siRNA complexes lastly recruits DRM2 to methylate the target DNA (Duan et al., 2015; Li et al., 2006; P. Raja et al., 2014). Furthermore, in *A. thaliana*, in addition to 24-nt hc-siRNAs, RDR6-dependent 21-nt siRNAs which originate from trans-acting siRNA (TAS) loci have the ability to recruit AGO4, a process that directs DNA methylation at the TAS loci (Wu et al., 2012). 24 nt-long miRNAs (lmiRNAs), which are generated from the actions of DCL1, in conjunction with AGO4, have the ability to direct DNA methylation in *trans* at the targeted gene site, thereby resulting in TGS. DNA viruses accumulate in the nuclei of infected plants where AGO4 binds to the generated viral siRNA, forming a complex. The AGO4/siRNA complex is also exported into the cytoplasm and processed as described above. In addition to AGO4, given the close phylogenetic relationship of AGO6 and AGO9 to AGO4; AGO6 and AGO9 have also been reported to mediate de novo DNA methylation by recruiting 24-nt siRNAs (Nuthikattu et al., 2013; Pontier et al., 2012).

AGO4 is the major AGO protein involved in hc-siRNA action, that directs DNA methylation via the RdDM pathway (Li et al., 2006; Qi et al., 2009). RdDM mechanisms have evolved in part to silence foreign or repetitive DNA and, not surprisingly, this mechanism also functions against plant DNA viruses. However, despite the existence of several RdDM-associated AGO proteins, only AGO4 has been implicated in antiviral mechanisms against DNA viruses, likely because it has a broad and constitutive expression pattern. As would be expected, AGO4 inhibits DNA viruses through a methylation-mediated mechanism (P. Raja, Jackel, Li, Heard, & Bisaro, 2014; Priya Raja et al., 2010, Wang et al., 2019). Surprisingly, although AGO4 is best characterized for its function in the nucleus, it is also important in the defense against RNA viruses that have cytoplasmic replication strategies. Mutation of *ago4* allows for a higher accumulation of tobacco rattle virus (TRV) and BaMV, although AGO2 seems to be the more important AGO in these cases (Alazem et al., 2017; Ma et al., 2015). AGO4 is also reported to be required for resistance to the bacterial pathogen, *Pseudomonas syringae* infection (Agorio & Vera 2007). It has been reported that the loss of function in RdDM pathway components, upstream or downstream of AGO4 affects resistance to *P. syringae* pv. *tomato* DC3000 infection (Agorio & Vera 2007). However, it is interesting to note that for viruses, loss of other RdDM pathway components does not impair

resistance to plantago asiatica mosaic virus (PlAMV) infections, suggesting that AGO4 most likely has a function in defense independent of its role in RdDM pathway (Brosseau et al. 2016). Interestingly, in the presence of PlAMV, the localization of AGO4 shifted from being primarily in the nucleus to being mainly in the cytoplasm and a nuclear-localization deficient mutant of AGO4 was not compromised in its antiviral activity (Brosseau et al., 2016). Normally, AGO4 binds to hcsiRNAs in the cytoplasm (Ye et al., 2012), upon which a nuclear localization signal (NLS) is exposed and the complex is imported into the nucleus. Thus, PlAMV infection results in either an inhibition of AGO4 import into the nucleus or an active re-localization to the cytoplasm. The mechanism behind this is not clear, however, it may represent a kind of counter-counter defense, wherein RNA silencing is countered by the PlAMV, but this may in turn result in a mobilization of AGO4 by localizing to where it is able to target the viral RNA. AGO4 is also required in plant defense against DNA viruses, via the RdDM pathway, suggesting that DNA methylation by AGO4 is a mechanism used by plants to defend against DNA viruses (see below). For example, it has been reported that the DNA of a beet curly top virus (BCTV) VSR-defective mutant extracted from recovered plants is hyper methylated and that host recovery requires AGO4 (Raja et al. 2008).

AGO6 mutation partially suppresses TGS and affects DNA methylation at several RdDM target loci (Zheng et al., 2007). Compared to AGO4, AGO6 differs in expression pattern; AGO6 is predominantly expressed in the shoot and root apical as well as in dividing cells (Eun et al., 2011; Zheng et al., 2007). Studies have demonstrated that 21-22-nt siRNA, produced via RDR6 during RNA silencing of mRNAs, get incorporated into AGO6 directly. This then guides AGO6 to chromatin containing transposable elements for RdDM (McCue et al., 2015; Nuthikattu et al., 2013). Previous work has also shown that for DNA methylation, AGO6 and AGO4 are required at different stages or loci of DNA methylation (Duan et al., 2015).

AGO8 was previous thought to be a pseudogene due to a computational analysis prediction that the coding sequence of AGO8 contains splicing-inducing frame shifts; suggesting the formation of a nonfunctional protein (Takeda et al., 2008). However, AGO8 does appear to be functional in some species (Pradhan et al., 2017). In addition, a recent study showed that pre-meiotic ovules of *ago4* and *ago9* mutant gametes overexpress AGO8, suggesting a possible role of AGO8 in

compensatory effect of gametophytic cell fate (Hernández-Lagana et al., 2016.) *A. thaliana* AGO9 is involved in controlling female gamete formation; by restricting specification of gametophyte precursors (Olmedo-Monfil et al., 2010). AGO9 was also shown to interact with 24-nt siRNAs derived from transposable elements and it silences transposable elements in female gametes in a non-cell autonomous manner (Olmedo-Monfil et al. 2010).

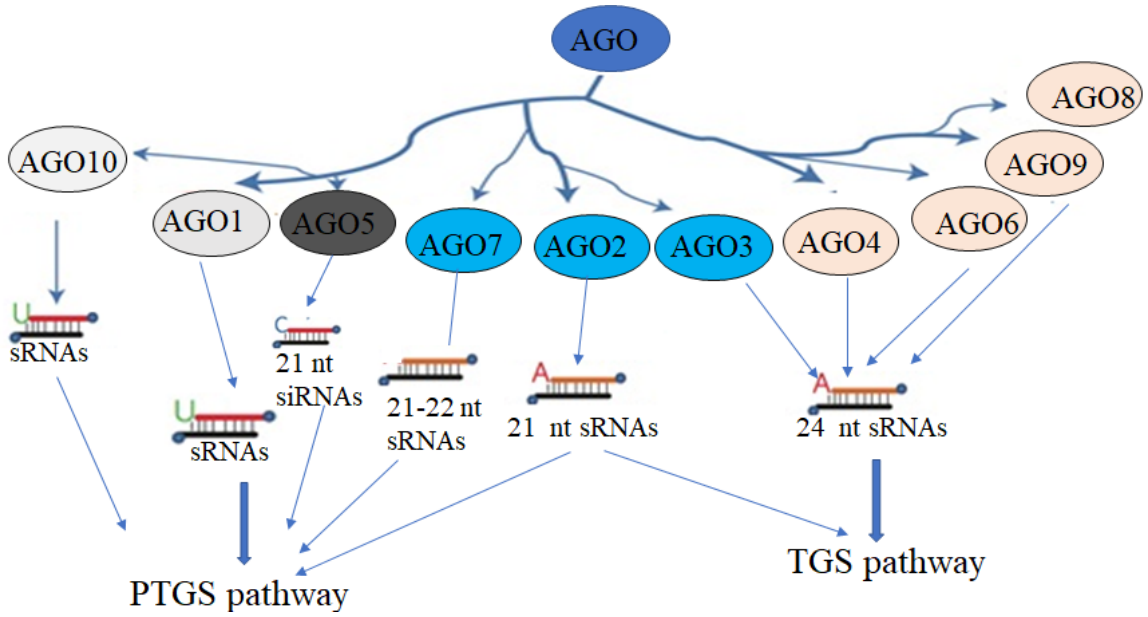

Figure 1.3. AGO proteins classifications and siRNA binding partners. Small RNA binding preference for AGO proteins and their downstream functions, using data from *A. thaliana* research. AGOs are colour coded and arranged with respect to their clades.

1.7 DNA viruses and Methylation-mediated defense.

In plants, the majority of studies that have investigated the role of RNA silencing during DNA virus infection uses geminiviruses (Inoue-Nagata et al., 2016). *Geminiviridae* is a large family of plant viruses that causes crop diseases of economic importance worldwide (Inoue-Nagata et al., 2016). Their genome consists of a circular single-stranded DNA (ssDNA) genome encapsulated in a twinned icosahedral particle and they replicate through a double-stranded DNA intermediate (dsDNA) (Inoue-Nagata et al., 2016). During infection by DNA viruses, DCL4 and DCL3-derived

vsiRNA initiate PTGS or TGS respectively (Csorba et al., 2015). DCL3-generated 24 nt vsiRNA following the HEN1-methylation are loaded into AGO4. While the silencing of RNA viruses predominantly takes place in the cytoplasm via PTGS mechanisms, silencing of DNA viruses occur in both the cytoplasm and the nucleus (Chellappan et al., 2005; Laufs et al., 1995).

Geminivirus-derived sRNAs of 21, 22 and 24-nt have been identified in infected hosts and all four DCLs in *A. thaliana* have been implicated in the production of geminivirus siRNAs (Priya Raja et al., 2014, 2008). *A. thaliana* methylation-deficient mutants are reported to be hypersusceptible to geminivirus infection. Although the viral dsRNA structures are accessible to all DCLs, a hierarchy exists between them and DCL3 has been reported to be crucial against DNA viruses (Akbergenov et al., 2006; Qu et al., 2008; Csorba et al., 2015). *A. thaliana dcl3* mutant are unable to recover from geminivirus infection when compares to wild-type, *dcl2* and *dcl4* (Raja et al., 2014). Furthermore, studies of virus induced gene silencing (VIGS) triggered by CaLCuV, a geminivirus, have shown that DCL2 and 3, RDR6, HEN1 and SGS3 are required to convert geminivirus-derived transcripts into dsRNAs, which induces RNA silencing of host mRNAs (Akbergenov et al. 2006; Muangsan et al. 2004). In addition, a number of VSRs encoded by these geminiviruses are able to suppress PTGS (Buchmann et al., 2009). These include the AL2-like proteins from african cassava mosaic virus and tomato yellow leaf curl virus, P6 protein from cauliflower mosaic virus (CaMV) and L2 of BCTV (Raja et al., 2008).

1.8 Detailed roles of AGO proteins in plant-pathogen interaction

1.8.1. RNA Silencing as a defense mechanism against viruses

While RNA silencing is mostly involved in endogenous gene regulation, given its ability to degrade dsRNA and target homologous ssRNA, it also functions as a defense mechanism against virus infection (Figure 1.4). Most plant viruses have an RNA genome and/or generate dsRNA from replication intermediates, local self-complementary regions of the viral genome, or through the action of host RNA-dependent RNA polymerase (RDR) on viral RNA (vRNA) templates (Donaire et al., 2009; Qi et al., 2009; Szittya et al., 2010). Like endogenous targets, viral dsRNA can also

be processed by DCL proteins into viral small interfering RNAs (vsiRNA). As outlined above, RNA silencing encompasses multiple related gene-regulating phenomena and different RNA silencing components have undergone amplification and diversification, like AGO proteins. Many of these were initially identified in studies investigating transgene silencing and/or developmental regulation of endogenous gene expression (Adenot et al., 2006; Bohmert et al., 1998; Fagard et al., 2000; Lynn et al., 1999; Moussian et al., 1998; Zheng et al., 2007; Zilberman et al., 2003) and subsequently tested for the role the play in virus defense. Thus, it begs the question: have AGOs become specialized to target viruses in different plants and if so, which AGOs are able to do so.

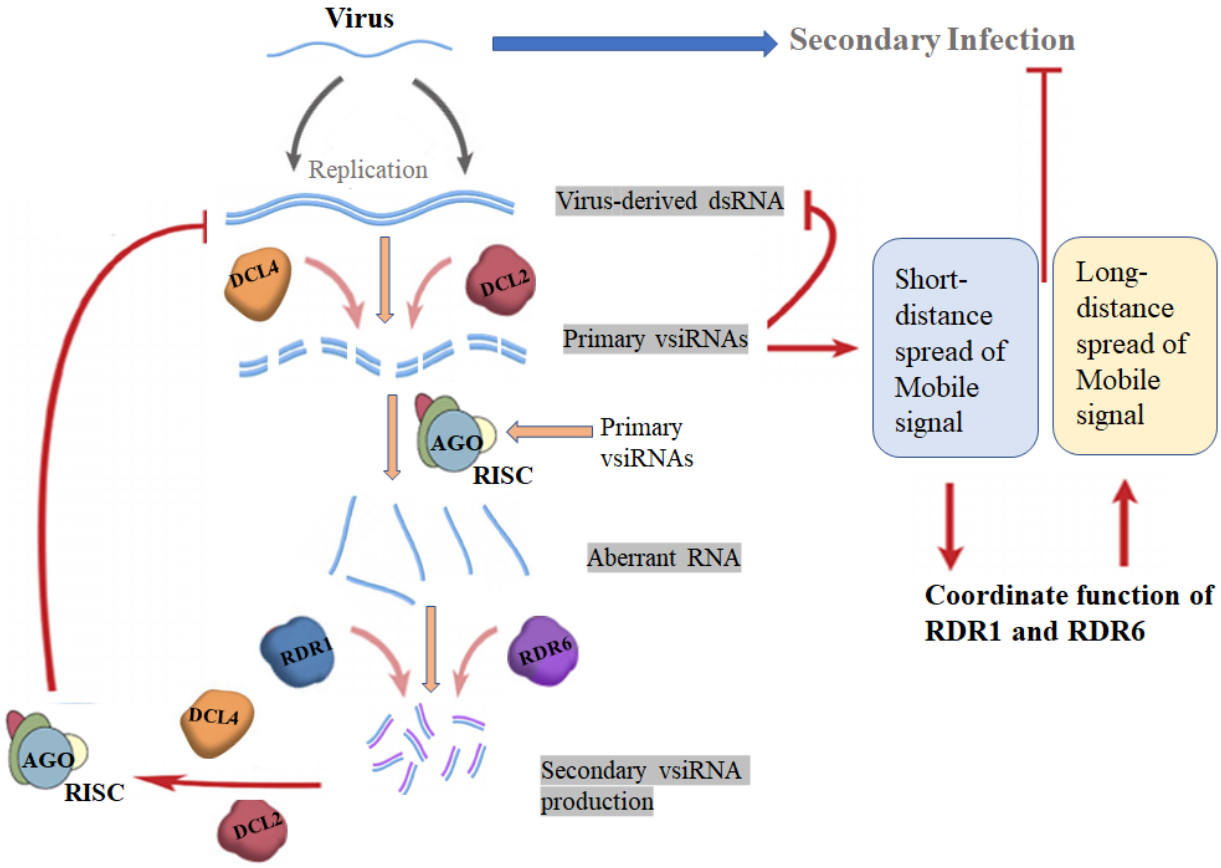

Figure 1.4 Schematic representation of RNA silencing during viral infection. A Model for plant antiviral defense based on RNA silencing. Virus-derived dsRNA structures are recognized by DCL4 and DCL2, which leads to the generation of DCL-dependent vsiRNAs. The vsiRNAs are loaded into the RISC complexes, where AGO proteins are contained. The vsiRNA-loaded RISC complexes targets viral transcripts for cleavage. Aberrant RNAs are also generated, which are targeted by RDR1 and RDR6. The RDR-dependent secondary vsiRNAs target viral regions

that are distant from the sites of primary vsiRNA processing. Modified from (Willmann et al., 2011).

1.8.2 WHICH AGOs ARE ANTIVIRAL?

The study of antiviral RNA silencing *in planta* comes with inherent challenges since this requires the use of a virus and compatible host. However, if a virus can infect a given host, this usually means that it has overcome the host's RNA silencing mechanisms, most likely through the action of its VSR. This experimental constraint can be overcome by using VSR-defective mutant viruses to infect mutant plants to determine what genetic ablations can compensate for the lack of VSR activity. This approach has been very informative but since viral proteins are often multi-functional, it is not possible for all viruses. Alternatively, one can use wild-type (WT) viruses and assess for increased virus accumulation in mutant plants. Table 1 lists studies showing genetic evidence for the involvement of different AGO proteins, as assessed by increased virus accumulation of either WT or VSR-defective viruses in mutant plants. Early genetic studies and hypotheses regarding antiviral RNA silencing focused on AGO1 due in part to the order in which different AGO proteins were characterized. Although many initial insights into RNA silencing phenomena came from the study of plant-virus interactions, most of the first genetic screens were designed to identify RNA silencing components affecting the post-transcriptional silencing of endogenous genes or transgenes. These led initially to the characterization of AGO4 and AGO1 (Bohmert et al., 1998; Zilberman et al., 2003). The first AGO protein shown to virus infection was AGO1, with the report that a hypomorphic *ago1* mutant was hyper-susceptible to CMV (Morel et al., 2002). This was one of the only AGO mutants available at the time and subsequent studies reported that *ago1* mutants permitted increased accumulation of brome mosaic virus (BMV), as well as VSR-defective variants of CMV and TCV, suggesting an important function for AGO1 in antiviral RNA silencing (Dzianott, Sztuba-Solińska, & Bujarski, 2012; Qu, Ye, & Morris, 2008). In *Nicotiana benthamiana*, silencing of *AGO1* attenuates symptom recovery in plants infected with tomato ringspot virus (ToRSV) (Ghoshal and Sanfaçon, 2014). Likewise, *AGO1* knockdown in rice (*Oryza sativa*) permits increased accumulation of rice dwarf phytoreovirus (RDV) and rice stripe virus (RSV) (Wu et al., 2015). Thus, AGO1 appears to play a role in multiple plant-virus interactions. However, it should be noted that studies with AGO1 are often confounded by the

severe developmental phenotypes exhibited by *ago1* mutants and that AGO1 is involved in the regulation of large numbers of endogenous genes (Baumberger & Baulcombe, 2005; Bohmert et al., 1998; Kidner & Martienssen, 2005; Morel et al., 2002), some of which could indirectly affect virus infection.

The availability of mutants for all AGO-encoding genes in *A. thaliana* has allowed for systematic analyses to identify which AGO proteins have antiviral activity (Table 1). This approach has enabled multiple groups to identify AGO2 as being involved in resistance against a range of viruses, including, PVX, CMV, TCV, TRV, TuMV, PlAMV and BaMV, suggesting a broad involvement of AGO2 in defense against viruses (Jaubert et al. 2011; X.-B. Wang et al. 2011; Harvey et al. 2011; Cabonell et al. 2012; Ma et al. 2015; X. Zhang et al. 2012; Brosseau et al. 2016; Hernan Garcia-Ruiz et al. 2015; Alazem et al. 2017). Multiple studies have demonstrated a role for AGO2 in antiviral defense in *N. benthamiana* as well. When *NbAGO2* is silenced by virus-induced gene silencing (VIGS), several *Tombusviruses* (TBSV, cymbidium ringspot virus [CymRSV], carnation Italian ringspot virus [CIRSV] and cucumber necrosis virus [CNV]) accumulate to higher levels (Odokonyero et al., 2015) and plants no longer recover from infection by a VSR-defective version of TBSV (Scholtof et al., 2011). Likewise, CRISPR-generated *N. benthamiana ago2* knockout plants are more susceptible to PVX and TuMV. The same mutants also showed more symptoms when infected with TCV, VSR-deficient CIRSV and CymRSV (Ludman et al., 2017a). Given the similarities of AGO7 and AGO3 with AGO2, it might be predicted that these proteins might also be involved in antiviral defense. However, AGO7 has been implicated in plant-virus interactions only to a small extent. *A. thaliana ago7* mutants show some increase in susceptibility to BaMV as well as attenuated versions of TCV-GFP and TuMV, albeit to a limited degree (Alazem et al., 2017; Garcia-Ruiz et al., 2015; Qu et al., 2008). In *N. benthamiana*, VIGS of AGO7 caused a subtle increase in susceptibility to TBSV, sunn-hemp mosaic virus (SHMV), and foxtail mosaic virus (FoMV) (Odokonyero et al., 2017). Likewise, AGO3 has only been shown to play a relatively minor role in curtailing infection of one virus, BaMV (Alazem et al., 2017). Thus, AGO2 appears to have evolved to play a major role in anti-viral defenses (Table 1.1).

In addition to genetic analyses, antiviral activities of AGO proteins can also be assessed using, *Agorobacterium*-mediated transient expression assays (agroinfiltration), wherein AGO proteins are co-expressed in *N. benthamiana* leaves infectious clones of a virus. This approach has also identified AGO2 as a major antiviral AGO protein, as well as other AGO proteins (Alazem et al., 2017; Brosseau and Moffett, 2015; Fátyol et al., 2016). Indeed, this approach can be used as a relatively rapid assay to identify antiviral proteins that may not be evident in genetic assays. For example, *A. thaliana* plants lacking *DCL2*, *DCL2* and *DCL4* (triple dicer: TD) are more susceptible to PVX than *ago2* mutants, the only single *ago* mutant that allows systemic infection by PVX (Brosseau and Moffett, 2015; Jaubert et al., 2011). However, in a transient assay in *N. benthamiana* leaves, both AGO2 and AGO5 decreased virus accumulation. Furthermore, AGO5 was induced mainly in the upper non-inoculated leaves of virus-infected plants and a double *ago2 ago5* mutant showed a level of susceptibility to PVX equal to the TD mutant (Brosseau and Moffett, 2015). These data showed an interaction between both proteins in a spatiotemporal manner, with AGO2 functioning as the first layer of defense against PVX and AGO5 acting as a second layer against subsequent systemic movement and infection (Brosseau & Moffett, 2015). The degree to which this paradigm applies to other viruses has yet to be extensively studied, although it is notable that VIGS of an AGO5 homologue in *N. benthamiana* led to an accumulation of VSR-defective TBSV (Odokonyero et al., 2017).

AGO4 appears to inhibit DNA viruses through a methylation-mediated mechanism (P. Raja, Jackel, Li, Heard, & Bisaro, 2014; Priya Raja et al., 2010, Wang et al., 2019) and is targeted by the VSRs of multiple DNA viruses (Hernan Garcia-Ruiz et al., 2015; Li & Wang, 2019). Surprisingly, although AGO4 is best characterized for its function in the nucleus, it has also been shown to be important in the defense against RNA viruses, which have cytoplasmic replication strategies. Mutation of *ago4* allows for a higher accumulation of TRV and BaMV, although AGO2 seems to be the more important AGO in these cases (Alazem et al., 2017; Ma et al., 2015). However, *ago4* mutants are much more susceptible to PlAMV, with AGO2 playing a lesser role (Brosseau et al., 2016).

Table 1.1: List of AGOs reported to have antiviral activities, as well as the wild-type virus and/or VSR-defectived mutant version (X) used in the corresponding study.

AGO protein	Plant species	Virus			References
			Wild-type virus	Mutant Virus	
AGO1	*Arabidopsis thaliana*	CMV	X	X	(Morel et al., 2002; Wang et al., 2011)
		BMV	X		(Dzianott et al., 2012)
		TCV		X	(Qu et al., 2008; Azevedo et al., 2010; Zheng et al., 2019)
	Oryza sativa	SRBSDV		X	(Xu and Zhou, 2017)
		RDV	X		(Wu et al., 2015)
		RSV	X		(Wu et al., 2015)
	Nicotiana benthamiana	ToRSV	X		(Ghoshal and Sanfaçon, 2014)
AGO2	*Arabidopsis thaliana*	TRV	X		(Ma et al., 2015)
		BaMV	X		(Alazem et al., 2017)
		PVX	X		(Jaubert et al., 2011; Brosseau and Moffett; 2015)
		CMV		X	(Harvey et al., 2011; Wang et al., 2011)

	TCV	X		(Xiaofeng Zhang et al., 2012)
	TuMV		X	(Carbonell et al., 2012; Garcia-Ruiz *et al.*, 2015)
Oryza sativa	SRBSDV	X	X	(Xu and Zhou, 2017)
Nicotiana benthamiana	TBSV	X		(Odokonyero et al., 2015; Scholthof et al., 2011)
	ToRSV	X		(Paudel et al., 2018)
	PVX	X		(Ludman et al., 2017; Odokonyero et al., 2015)
	TuMV	X		(Ludman et al., 2017)
	TCV	X		(Ludman et al., 2017)
	CIRSV	X	X	(Ludman et al., 2017; Odokonyero et al., 2015)

		CNV	X	X	(Ludman et al., 2017; Odokonyero et al., 2015)
		TMV	X		(Diao et al., 2019)
		CymRSV	X	X	(Ludman et al., 2017; Odokonyero et al., 2015)
AGO3	*Arabidopsis thaliana*	BaMV	X		(Alazem et al., 2017)
AGO4	*Arabidopsis thaliana*	BaMV	X	X	(Alazem et al., 2017)
		BCTV	X		(P. Raja et al., 2014, 2008b)
		TRV	X		(Ma et al., 2015)
		PlAMV	X		(Brosseau et al., 2016)
AGO5	*Arabidopsis thaliana*	PVX	X		(Brosseau and Moffett, 2015)
		TuMV		X	(Garcia-Ruiz et al., 2015)
NbAGO5L	*Nicotiana benthamiana*	TBSV		X	(Odokonyero et al., 2017)
AGO7		BaMV	X		(Alazem et al., 2017)

	Arabidopsis thaliana	TuMV		X	(Garcia-Ruiz et al., 2015)
		TCV	X		(Qu *et al.*, 2008)
	Nicotiana benthamiana	TBSV	X		(Odokonyero et al., 2017)
		SHMV	X		(Odokonyero et al., 2017)
		FoMV	X		(Odokonyero et al., 2017)
		TuMV		X	(Garcia-Ruiz et al., 2015)
AGO10	*Arabidopsis thaliana*	TuMV		X	(Garcia-Ruiz et al., 2015)
AGO18	*Oryza sativa*	RDV, RSV	X		(Wu et al., 2015)

1.8.3 Viruses and Their VSRs: AGO/VSR Interaction

Most plant viruses have been reported to produce proteins, known as viral suppressors of RNA silencing (VSRs), that suppress components of their host plants' RNA silencing defense pathways (Vance & Vaucheret 2001). Most plant viruses encode at least a VSR while others encode multiple VSRs (Roth et al., 2004). It is interesting to note that the majority of plant VSRs do not share obvious sequence or structural similarity across viral groups and families (Qu and Morris, 2005; Roth et al., 2004). However, VSRs have been identified as host range determinants (Qu and Morris, 2005). Suppressor activity has been identified in structural as well as in non-structural proteins involved in almost every viral function (Carbonell and Carrington, 2015; Fang and Qi, 2016). VSRs have the ability to target some of the effectors of the silencing pathway such as the

recognition of viral RNA, RNA targeting and amplification and RISC assembly (Qu and Morris, 2005).

The majority of plant VSRs have been reported to sequester primary and secondary siRNA and prevent their incorporation into the RISC (Anandalakshmi et al. 2000; Lakatos et al. 2004; Silhavy et al. 2002). For instance, P14 of pothos latent aureusvirus and P38 of TCV are two VSRs that have been shown to inhibit the processing of dsRNA to siRNAs by binding dsRNAs in a size independent manner (Mérai et al., 2005). Some studies have also shown that the P38 protein binds AGO1 thereby inhibiting its activity (Mérai et al., 2005). In addition, some VSRs such as the P6 of cauliflower mosaic virus (CaMV) inhibits the processing of vsiRNA by interacting with dsRNA-binding protein 4, a protein needed for DCL4 function (Haas et al., 2008). A common strategy used by VSRs is the sequestration of siRNAs, thereby preventing the assembly of the RISC complex. The P19 of tombusviruses is a good example of a VSR that prevents RNA silencing by siRNA sequestration because it has a high affinity for siRNA (Lakatos et al. 2004; Várallyay & Havelda 2013; Kontra et al. 2016). The 2b protein of CMV is a well-studied VSR and has been shown to physically inhibit the slicing activity of AGO1 by interacting with its PAZ domain (Fang et al., 2016; Feng et al., 2013; Duan, & Guo, 2013). The 2b protein also interacts with AGO4 and inhibits systemic silencing by suppressing RdDM (González et al., 2012, 2010; Goto et al., 2007; Hamera et al., 2012; Zhang et al., 2006) and binds long dsRNA to limit the productions of viral secondary siRNAs (Duan et al., 2012). Therefore, the 2b protein is a multi-effector of silencing inhibition.

Potyviruses constitute the largest group of plant RNA viruses (Adams et al., 2005). The VSR of the viruses in this genus are called helper-component protease (HC-Pro). HC-Pro is also a multifunctional protein involved in many mechanisms of viral replication and life cycle (Adams et al., 2005). It was also the first identified VSR in plants (Adams et al., 2005). Although it is a well-characterized VSR, its mechanism of action is not fully understood. Work by Ivanov et al., (2016) suggests two distinct and overlapping mechanisms by which HC-Pro suppresses RNA silencing. The first proposed mechanism involves an interaction between HC-Pro and AGO1, with the latter inhibiting the RISC translational inhibition of viral RNAs (Ivanov et al., 2016a). In the

second putative mechanism, HC-Pro in complexes with other viral and host proteins, inhibits key enzymes of the methionine cycle and as a result, methylation of sRNAs by HEN1 is prevented, thereby leading to suppression of RNA silencing.

Potexviruses possess a triple gene block (TGB) of three proteins that are involved in virus movement and establishing replication structures (Ha and Kim, 2014). These TGB are named TGB1, 2 and 3. The TGB1, or P25 protein, of several potexviruses has been shown to have VSR activity (Senshu et al., 2017). Similar to the 2b protein of CMV, PVX P25 protein interacts with *A. thaliana* AGOs 1, 2, 3, 4 and 5 when these proteins are transiently expressed *in N. bethamiana* (Brosseau and Moffett, 2015; Chiu et al., 2010). Similar to TCV, AGO1 and AGO7 have also been reported to target PVX in the absence of its VSR, P25 protein (Brosseau and Moffett 2015). PVX P25 is considered a weak VSR and PVX is a virus that is unable to replicate in the non-host *A. thaliana* unless the silencing pathway is inhibited (Jaubert et al., 2011; Powers et al., 2008; Senshu et al., 2017). The P25 protein of PlAMV is also known to target host components involved in RNA silencing amplification, including RDR6, SGS3 (Okano et al., 2014).

The sweet potato mild mottle ipomovirus (SPMMV) suppressor P1 also has GW/WG-like motif that binds and suppresses *A. thaliana* and *N. benthamiana* AGO1-RISC function (Giner et al., 2010). A recent study demonstrated that alfalfa dwarf virus (ADV) VSR phosphoprotein (P) inhibits the activity of programed AGO1-RISC and AGO4-RISC, although this VSR does not contain GW/WG motifs (Bejerman et al., 2016). Even though AGO1 and AGO4 were the main AGO reported in these studies, it does not rule out the putative effect of those latter VSRs in other AGOs of different plant species. The polerovirus VSR P0 and PlAMV P25 are strong VSRs and that induce the degradation of many AGO proteins from *A. thaliana.* In a number of studies, P0 targeted all the *A. thaliana* AGO tested; AGO1, AGO2, AGO4, AGO5, AGO6 and AGO9 (Bortolamiol et al., 2007; Csorba et al., 2010; Derrien et al., 2012). Aside from the strong effect of the P25 of PlAMV in the suppression of RDR6/SGS3 RNA-silencing amplification, the P25 protein has also been reported to target AGO1, 6, 7, 9 and 10 (Brosseau et al., 2016; Okano et al., 2014). When all the AtAGOs were tested *in trans,* AGO2, 3, 4 and 5 were the ones that were not degraded by P25 and possess antiviral activities against this RNA virus.

As discussed above, all AGO proteins seem to possess the ability to target vRNA (Table 1) if it is not protected by a VSR (Brosseau and Moffett, 2015). However, a significant portion of genetic studies have used viruses mutated for their ability to suppress RNA silencing. Certain VSRs appear to target specific AGO proteins, however, the basis of these specificities remains unclear. The remaining part of this introduction will explore the three viruses used in this study and their characteristics.

1.9 Viruses used in this study

1.9.1 Cucumber Mosaic Virus

CMV belongs to the genus *Cucumovirus,* family *Bromoviridae* (Pita et al., 2015). CMV is an extremely successful virus as it infects more than 1,000 species of plants, including monocots and dicots (Jacquemond, 2012; Montes et al., 2019; Pagán et al., 2010a). CVM has been isolated in all parts of the world and numerous strains have been identified and characterized (Roossinck, 2002). Therefore, due to its evolutionary elasticity, CMV is an excellent model system for studying RNA virus and plant interaction (Jacquemond, 2012; Montes et al., 2019; Pagán et al., 2010a), and has been used as a model for experimental evolution studies (Pita et al., 2015).

CMV is a tripartite plus sense RNA virus with a divided genome (Palukaitis et al., 1992). The genomic RNAs are packaged into separate particles to allow the packaging of large genomes into a very simple virion. The natural transmission of CMV occurs by aphids in a non-persistent manner (Palukaitis et al., 1992), which most likely ensures a multiplicity of infection sufficient to reliably establish infection, and by seeds (Elsharkawy et al., 2013; Hily et al., 2014). CMV contains five open reading frames (ORFs) (Figure 1.5). The 1a and 2a ORFs are encoded on RNAs 1 and 2 respectively and they are the viral components of the replicase. The 2b ORF, a gene overlapping the 2a ORF, is expressed from a sub-genomic RNA, RNA 4A (Roossinck, 2001) and encodes a suppressor of posttranscriptional gene silencing (Roossinck, 2001). RNA 3 encodes the 3a protein

which is the viral movement protein and the coat protein (CP) is expressed from the sub-genomic RNA 4 (Roossinck, 2001). Both proteins are required for viral movement (Roossinck, 2001).

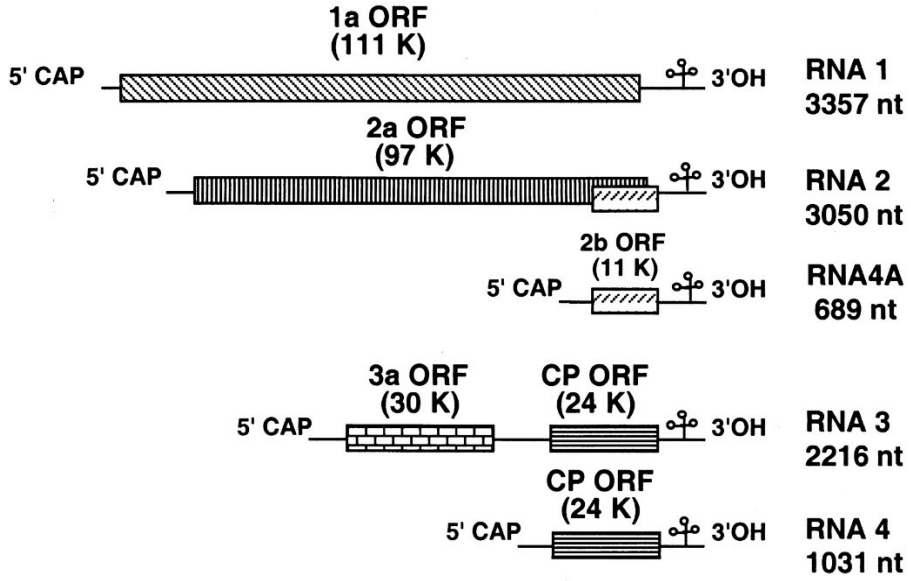

Figure 1.5. Genome organization of CMV. Nucleotide (nt) numbers and the sizes of encoded proteins are given in kilodaltons (K) (obtained from Roossinck, 2001).

1.9.2 Potato Virus X

PVX is the type member of the potexvirus group of plant plus-strand RNA viruses (Lico et al., 2015). It has been a useful virus for model systems that study various aspects of virus infections and how these features can be exploited for biotechnology applications. PVX is transmitted mechanically and its main hosts are *Solanaceae* (Lico et al., 2015). PVX has also been reported to be have important economic impacts and its effects can be worsened by co-infection with other viruses (Syller, 2012). PVX contains a relatively small, single-stranded genomic RNA that is functionally monocistronic. PVX encodes 5 ORFs and the product of ORF1 encodes the PVX replicase, which is responsible for RNA synthesis. PVX requires four ORF products for cell-to-cell and long-distance movement in infected plants (Jens Tilsner et al., 2013; Lico et al., 2015; Verchot-Lubicz et al., 2010). Three of these are encoded by the overlapping ORFs 2-4, which are called the 'triple gene block' (TGB) (Lico et al., 2015; Solovyev et al., 2012). The 25 kDa protein

(called P25 or TGBp1), encoded by ORF2 is known to function as a suppressor RNA gene silencing in the host plant (Senshu et al., 2009; Solovyev et al., 2012). In addition, TGBp1 has been shown to gate the plasmodesmata (PD) and localize to them in infected cells (Howard et al., 2004; Samuels et al., 2007), however, it does not target the PD itself. TGBp1 has been hypothesized to be recruited to the PD by the other two TGB proteins, as is seen in other TGB-encoding viruses (Erhardt et al., 2000; Verchot-Lubicz et al., 2010). TGBp2 and TGBp3 are small transmembrane proteins that have been reported to co-localize in the ER. TGBp2 is the most conserved of the TGB proteins and it has been shown to affect the permeability of the PD. Although TGBp3 is not absolutely required for cell-to-cell movement, it has been shown to be important for efficient movement, and may function intracellularly by facilitating the trafficking of TGBp2 and it is usually reported as the PD targeting factor (Lee et al., 2010; Lough et al., 2006; Schepetilnikov et al., 2005; Solovyev et al., 2000). All three TGB proteins as well as the capsid protein (CP) are ultimately required for PVX movement. TGBp2 and 3 have been shown to remodel the endoplasmic reticulum (ER) at the PD orifice into 'caps' that harbor the replicase and vRNA, all as an effort to enable the widespread of virus infection within the host plant (Tilsner et al., 2013).

Figure 1.6 PVX genome. RdRP: RNA-dependent RNA polymerase (replicase), indicating the overlapping reading frames of the triple gene block proteins (TGBp) modified from (Tilsner et al., 2013).

1.9.3 Turnip Mosaic Virus

Turnip mosaic virus (TuMV) is a member of the genus Potyvirus and it has been reported to be the most widespread and damaging virus that infects both crop and ornamental species of the *Brassicaceae* family worldwide (Nguyen et al., 2013). *A. thaliana* is readily infected by TuMV and TuMV is the only potyvirus regularly found naturally infecting cruciferous plants. TuMV has

been shown to be the best adapted potyvirus to *A. thaliana* and many have had an impact on *A. thaliana* evolution/adaptation (Jenner et al., 2002). TuMV was ranked second to CMV as the most important virus infecting field-grown vegetables in 28 countries and regions (Walsh and Jenner, 2002). TuMV causes severe morphological and developmental defects in the C24 ecotypes of *A. thaliana*, including stunted and deeply serrated leaves; flowers have narrow sepals, split carpels and aborted anthers and the plants are sterile (Jenner et al., 2002).

The TuMV genome consists of a positive-sense single strand RNA molecule that is approximately 10,000 nucleotides in length (Nguyen et al., 2013). The 5'-terminus of the genomic RNA is capped with a single covalently attached molecule of the virus-encoded VPg protein. The 3'-terminus consists of a polyA tail of variable length. The genome encodes a long open reading frame (ORF) and another relatively short ORF that results from RNA polymerase slippage in the P3 coding sequence (Olspert et al., 2015; Revers and García, 2015). Upon translation, these two polyproteins are proteolytically processed by three viral protease domains into 11 mature viral proteins (Cheng and Wang, 2017). More recently, a short ORF, termed PISPO (pretty interesting sweet potato potyvirus ORF), was reported to result from transcriptional slippage, and was found to be embedded in the P1 coding regions (Mingot et al., 2016). The HC-Pro and VPg of TuMV have been shown to be VSRs (Kasschau and Carrington, 1998).

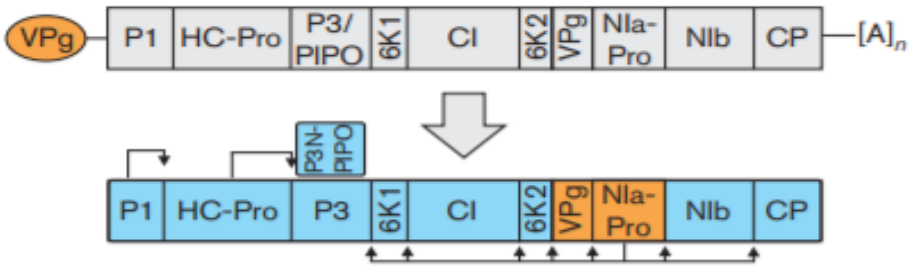

Figure 1.7 Schematic diagram of the TuMV genome. This schematic diagram shows the genome of potyvirus (designed from Jenner et al., 2000; Walsh an Jenner, 2002 and obtained from (Ivanov et al., 2014)). The genome of potyviruses is a positive-sense ssRNA molecule covalently linked to VPg at the 59 end and polyadenylated at the 39 end. The genome is translated into a single polyprotein, which is subsequently processed by virus-encoded proteases into individual

proteins. The P3N-PIPO protein is produced from a separate small ORF. Proteolytic sites are marked with arrows indicating the names of the corresponding proteases. Viral RNA is shown in grey and translated proteins in light blue. Abbreviations: UTR, untranslated region; P1, protein 1; HC-Pro, helper component protease; P3, protein 3; 6K1, 6kDa protein 2; VPg, virus-encoded genome-linked protein; NIa, nuclear inclusion protein a; NIb, nuclear inclusion protein b; CP, coat protein.

1.10 Purpose and Objectives of the projects.

1.10.1 Intra-species variation in *Arabidopsis thaliana AGO2* alleles and viral infection

Work in our lab investigating natural variation in the *AGO2* gene has shown that the *AGO2* allele found in the *A. thaliana* wild-type Columbia-0 (Col-0) accession confers resistance to potato virus X (PVX), whereas the *AGO2* allele found in the C24 accession does not (Brosseau et al., 2019). Based on these observations, we hypothesized that naturally occurring wild accessions that possess a Col-0-like *AGO2* will be resistant to PVX and other viral infection, whereas, wild accessions that possess a C24-like *AGO2,* will be susceptible to these viruses. Our objective was to: 1) Investigate the role of Col-0 *AGO2* gene in viral resistance; 2) Establish a non-defense function for the C24 *AGO2* that might explain its persistence in nature. To pursue these objectives, we utilized a population of Iberian Peninsula *A. thaliana* wild accessions and recombinant inbred lines (RILs). The former accessions have been reported to possess differing degrees of resistance to CMV, as well as well-characterized life history trait differences (flowering time, seed weight and inflorescent weight). Iberian Peninsula wild accessions were challenged with PVX, CMV and TuMV and their response to the viruses were assessed. The *AGO2* sequence of these accessions was also genotyped. For this study, we hypothesized that regardless of the background of the wild accessions and RILs, plants possessing the Col-0-like *AGO2* would be resistant to PVX and other viral infections while plants containing the C24-like *AGO2* would be susceptible. Certain life history traits were also measured in the wild accessions as well as in the RILs. By doing this, we investigated the role of both *AGO2* alleles on growth and development in the presence and absence of viruses. The findings of this study will help understand how natural variation of a gene within

a species could determine the outcome of viral resistance and the role that a viral susceptible allele plays for other life history traits in nature.

1.10.2 Differences between *Arabidopsis thaliana* AGO2 and *Nicotiana benthamiana* AGO2.

The first half of this project is heavily based on preliminary work from the Moffett lab (Brosseau et al., 2019). *N. benthamiana* AGO2 and *A. thaliana* AGO2 were transiently co-expressed with PVX. The expression levels of PVX and both AGO proteins were then evaluated. A decrease in the accumulation of *N. benthamiana* AGO2 in the presence of PVX-GFP was observed, whereas, the accumulation level of *A. thaliana* AGO2 remained unchanged (Brosseau et al., 2019). This result explains to a large extent why *N. benthamiana* AGO2 has low antiviral properties against PVX. The experiment further showed that the decrease in accumulation of AGO2 from *N. benthamiana* was dependent on the presence of the P25 protein of PVX. Based on these findings the objective of this study included the transformation of *N. benthamiana* plants with *A. thaliana* AGO2 and the investigation of the response of these transgenic plants to PVX infection. We hypothesized that *A. thaliana* AGO2 expressed in *N. benthamiana* would make these transgenic plants resistant to PVX infection.

CHAPTER 2

Genetic variation in *AGO2* determines susceptibility to virus infection and other life history traits.

For this article, the natural variation of *A. thaliana AGO2* alleles was investigated in Iberian Peninsula wild accessions of *A. thaliana*. Firstly, the *AGO2* gene from these accessions were sequenced and characterized. Wild accessions were subsequently inoculated with three viruses: potato virus x (PVX), turnip mosaic virus (TuMV) and two strains of cucumber mosaic virus (CMV). The natural polymorphisms in *A. thaliana AGO2* in the Iberian Peninsula wild accessions determined susceptibility to PVX and to TuMV but not to CMV. Using recombinant inbred lines (RILs), these results were validated. We also characterized certain life history traits in the wild accessions and in the RILs. Our results indicate that natural variation in *AGO2* is important for resistant to PVX and TuMV, but not for all viruses. Furthermore, we find that *AGO2* alleles that are less efficient in virus defense confer a reproductive advantage in the absence of virus. These results are the first to demonstrate the different roles for different *AGO2* variants and demonstrate a hitherto undescribed trade-off between fitness and pathogen defense.

For this article, AA, FGA and PM designed the experiments. AA, NM and CB performed the experiments. AA, FGA and PM wrote the manuscript. The manuscript will be submitted to the *PLoS Pathogens* scientific journal.

2. Genetic Variation in *AGO2* determines susceptibility to virus infection and reveals opposing fitness trade-off between reproduction and resistance in a naturally occurring population.

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2.1 ABSTRACT

RNA silencing is a major mechanism of constitutive antiviral defense in plants. *Arabidopsis thaliana* encodes four Dicer-like (DCL) proteins and ten Argonaute (AGO) proteins that are specialized to function in different RNA silencing-related mechanisms. We have previously shown that AGO2 plays an important role in protecting plants against viruses, including *Potato virus X* (PVX) in *A. thaliana.* In addition, we have observed natural variation in the *AGO2* genes of *A. thaliana* ecotypes and found that certain polymorphisms in *AGO2* correlate with the degree of susceptibility to PVX. Work presented in this study evaluated *A. thaliana* wild accessions collected in the Iberian Peninsula, previously characterized for CMV susceptibility. These accessions were inoculated with PVX, turnip mosaic virus (TuMV) and cucumber mosaic virus (CMV) and the outcome of infections were quantified. Results from this work revealed that the natural polymorphism found in *AGO2* is important for resistance against PVX and TuMV but does not correlate with susceptibility to CMV. Our results indicate that natural variation in *AGO2* can have important effects on infection for some, but not all viruses. We also show the trade-off that exists between virus susceptibility and reproduction, as an allele that causes hyper-susceptibility to virus infection offers a reproductive advantage.

2.2 Author summary

Understanding the roles of Argonaute proteins in innate defense against viruses is a growing field of study in plant-pathogen research. Therefore, it is important to investigate the roles of these AGO proteins in naturally occurring wild accessions during several viral infections. Here, we show that polymorphisms in the *AGO2* gene is a major determinant in conferring susceptibility to PVX and TuMV infection. In addition, this study identified a significant function for the C24 *AGO2* allele in nature. Although the C24 *AGO2* confers susceptibility to PVX and TuMV, it offers a reproductive advantage in the absence of virus infections. This project is novel as little is known about natural variation in AGO-encoding genes and how these variations might affect the RNA

silencing process and outcome. Our findings shed new light on the AGO2 plant protein and its effectiveness against a potexvirus and potyvirus.

KEYWORDS: Argonaute 2, *Arabidopsis thaliana,* wild accessions, fitness trade-off, natural variation, RNA silencing.

2.3 INTRODUCTION

In plants, one mechanism of antiviral defense is based on the RNA silencing pathway. RNA silencing refers to multiple related processes in which small RNA (sRNA) target nucleic acids for regulation/inhibition in a sequence-specific manner (Carbonell and Carrington, 2015; Fang and Qi, 2016; Meister, 2013). The mechanism of RNA silencing begins with long double-stranded RNA (dsRNA) originating from a transgene, a genetic element or viral intruder (Carbonell and Carrington, 2015; Csorba et al., 2015; Fang and Qi, 2016). These duplexes are recognized by a complex containing members of a protein family called dicer, or dicer-like (DCL), proteins (Carbonell and Carrington, 2015; Csorba et al., 2015; Szittya and Burgyán, 2013). DCL proteins cleave dsRNA into small RNAs (sRNA) that are usually 21 to 24 nucleotides in length (Pumplin and Voinnet, 2013). Sources of dsRNA can include viral genetic material, resulting in virus-derived small interfering RNAs (vsiRNAs) (Li and Wang, 2019). These vsiRNAs are loaded into an RNA induced silencing complex (RISC), which includes an Argonaute (AGO) proteins. AGO proteins possess endonucleolytic activity (Carbonell and Carrington, 2015; Omarov et al., 2016; Pumplin and Voinnet, 2013) and are guided to their target RNAs by the sRNA to which they are bound (Boualem et al., 2016; Li and Wang, 2019; Rosa et al., 2018).

The *A. thaliana* genome encodes ten AGO proteins, which have evolved to undertake specialized RNA silencing functions (Eamens et al., 2008; Mallory and Vaucheret, 2010). Several AGO proteins have been reported to play a role in antiviral RNA silencing (Carbonell and Carrington, 2015; Fang and Qi, 2016). AGO4 is the main AGO protein involved in antiviral silencing against DNA viruses (Raja et al., 2014; 2008) while for RNA viruses, AGOs 1 and 2 are the prominent antiviral AGOs with AGOs 3, 5, 7 and 10 having limited roles in antiviral defense (Alazem et al.,

2017; Brosseau et al., 2019; Brosseau and Moffett, 2015; Jaubert et al., 2011; Kontra et al., 2016; Odokonyero et al., 2015; Qu et al., 2008; Scholthof et al., 2011). Studies continue to show that AGO2 is an important AGO for defense against multiple viruses (Alazem et al., 2017; Brosseau et al., 2019; Brosseau and Moffett, 2015; Carbonell and Carrington, 2015; Ludman et al., 2017; Odokonyero et al., 2015; Zheng et al., 2019b). For example, it has been reported in a non-host viral interaction study that the most important AGO protein for defense against PVX infection is AGO2, as mutating *AGO2* led to infection of *A. thaliana* by PVX (Jaubert et al., 2011). Likewise, a systematic characterization of all *ago* mutants identified AGO2 as the AGO protein most important for defense against both WT and VSR-defective turnip mosaic virus (TuMV) (Garcia-Ruiz et al., 2015). Plant viruses have been reported to produce proteins, known as viral suppressors of RNA silencing (VSRs), that interfere with the activity of various RNA silencing components (Roth et al., 2004; Vance, 2001) and VSRs have been identified as host range determinants (Qu and Morris, 2005).

Natural variation within wild populations of *A. thaliana* has been used as a tool to identify the underlying mechanisms of plant development, biotic and abiotic stress tolerance, as well as adaptation to diverse environmental conditions (Koornneef et al., 2004; Manzano-Piedras et al., 2014; Méndez-Vigo et al., 2011, 2013; Pagán et al., 2010a; Picó et al., 2008). Investigation of natural variation in *A. thaliana* with respect to virus resistance has led to the identification of the NLR-encoding *HRT1* and *RCY1* genes, as well as the jacalin-encoding *JAX1* gene (Cooley et al., 2000; Takahashi et al., 2001; Yamaji et al., 2012). In addition, genetic variation has also been identified in the *A. thaliana RTM* genes, and these variations have been reported to influence the ability of *RTM* to restrict the long distance movement of potyviruses (Cosson et al., 2012). However, little is known about natural variation in plant genes encoding RNA silencing components, such as those encoding AGO proteins, and whether such variation might affect virus resistance and/or other traits.

Both CMV and TuMV infect natural populations of *A. thaliana* (Pagán et al., 2010a) and PVX has been demonstrated to infect certain wild accessions (Brosseau et al., 2019). The latter depends on the presence of one of two common *AGO2* alleles that is widespread among genetically and geographically diverse *A. thaliana* accessions (Brosseau et al., 2019). The *AGO2* allele found in

the Col-0 accession is representative of PVX-resistant accessions, whereas *AGO2* variants similar to that found in the C24 accession confer susceptibility (Brosseau et al., 2019). We investigated the intra-specific variations in the *AGO2* gene, using natural *A. thaliana* wild accessions and Recombinant Inbred Lines (RILs) (Törjék et al., 2008). This included twenty-seven natural *A. thaliana* accessions collected from diverse environments in the Iberian Peninsula region (Méndez-Vigo et al., 2013, 2011; Montes et al., 2019). Polymorphisms present within the *AGO2* genome were identified within these wild accessions, with most of the wild accessions possessing either a Col-0-like *AGO2* allele or a C24-like *AGO2* allele. In agreement with previous work, accessions that possess a Col-0-like *AGO2* allele were resistant to PVX infection whereas wild accessions that possess a C24-like *AGO2* were susceptible (Brosseau et al., 2019). We have extended these observations to show that the same *AGO2* polymorphisms are responsible for differences in susceptibility to TuMV, but not to CMV. Furthermore, by assessing life history traits such as rosette weight, seed weight and inflorescence weight of the wild accessions and RILs, we showed that plants that possess a C24-like *AGO2* allele produce more seeds than the plants with a Col-0-like *AGO2* in the absence of virus. Our results suggest a gain-of-function for C24-like *AGO2* alleles resulting in a trade-off between reproduction and defense.

2.4 RESULTS

2.4.1 Two polymorphisms in *AGO2* are widely distributed in natural populations.

To correlate virus resistance and *AGO2* polymorphisms, we sequenced the 5' end of the *AGO2* gene from twenty-seven *A. thaliana* Iberian Peninsula wild accessions. Encoded variants of AGO2 were classified as either Col-0-like, with an N-terminal sequence identical to that present in the Col-0 genome, or C24-like, possessing a substitution of an aspartic acid for a glycine at position 33 (33G, relative to Col-0), as well as small deletions of varying length in a GR-rich motif N-terminal to residue 33 (Brosseau et al., 2019). Of the twenty-seven accessions analyzed, eight encoded a Col-0-like allele and eighteen encoded a C24-like allele. In addition, one "rare" allele (Brosseau et al., 2019) was identified with a complete (Col-0 like) GR motif but encoding a glycine at residue 33 (33G; C24-like) (Table 2.1).

Table 2.1: Classification of individual Iberian Peninsula wild accessions. Accessions are classified based on the presence of either an aspartic acid (D) or a glycine (G) at residue 33 and on the presence or absence (+/-) of a deletion in the GR motif of the encoded AGO2 protein. All lines were also genotyped for the presence or absence (+/-) of a functional variant of the *JAX1* gene.

Wild Accessions	Residue 33	Deletion in GR motif	Presence of *JAX1*
Bis-9	G	-	-
Bis-2	G	+	-
Cdc-16	G	+	-
Mar-10	G	+	-
Pra-5	G	+	-
Pra-10	G	+	-
Qui-6	G	+	-
Qui-9	G	+	-
San-5	G	+	-
Moc-1	G	+	-
Mar-6	G	+	-
Gra-2	G	+	-
Gra-6	G	+	-
Leo-4	G	+	-
Mar-2	G	+	+
Bis-8	G	+	+
Mar-8	G	+	+
Qui-3	G	+	+
San-9	G	+	+
Pra-0	D	-	-
Leo-11	D	-	-
San-2	D	-	-
San-4	D	-	-
San-12	D	-	-
Moc-9	D	-	-
Leo-1	D	-	-
Leo-9	D	-	-
Col-0	D	-	-
C24	G	+	-

2.4.2 Col-0 AGO2 confers resistance to PVX infection in wild accessions.

To extend previous findings that Col-0-like AGO2 variants confer resistance to PVX (Brosseau et al., 2019), the twenty-seven wild accessions were challenged with PVX. Twenty-one days post inoculation (dpi), the accumulation of the PVX coat protein (CP) was assessed by immunoblotting. Consistent with previous results (Brosseau et al., 2019), it was observed that PVX CP did not accumulate in upper non-inoculated leaf tissues of any of the eight wild accessions with a Col-0-like *AGO2* allele (Figure 2.1). However, accumulation of PVX CP was detected in upper non-inoculated leaves of thirteen out of the eighteen wild accessions with a C24-like *AGO2*. The *JAX1* gene, which encodes a jacalin-type lectin protein, has been reported to confer resistance to multiple potexviruses (Yamaji et al., 2012). However, in a majority of accessions, a premature stop codon in the *JAX1* gene results in a nonfunctional allele (Brosseau et al., 2019; Yamaji et al., 2012). Therefore, we tested for the presence of a functional *JAX1* gene in these wild accessions. Interestingly, all five wild accessions with a C24-like *AGO2* that showed no accumulation of PVX CP in upper non-inoculated leaf tissues were positive for a functional *JAX1* gene (Figure 2.2a). The only wild accession that possessed the rare allele of *AGO2* also tested positive for PVX accumulation (Figure 2.1). Therefore, in the absence of *JAX1,* Col-0 AGO2 protein appears to be sufficient for restricting PVX infection (Figure 2.1). To further validate differential anti-viral activity of different *AGO2* alleles in resistance to PVX, RILs were used, as previously shown (Brosseau et al., 2019). We identified RILs that have genomic loci surrounding and including the *AGO2* gene that were exchanged between two parental lines (i.e. Col-0 genetic background with C24 *AGO2* and C24 genetic background with Col-0 *AGO2*) (Brosseau et al., 2019; Törjék et al., 2008) (Supplementary Fig. 2.1). RILs that possessed a Col-0-like *AGO2* allele (N35, N62, and M39) showed no accumulation of PVX in upper non-inoculated tissues whereas RILs that possessed a C24-like *AGO2* (M44, M41 and N37) (Supplemental Fig. 1) showed accumulation of PVX in upper non-inoculated tissues, regardless of the genetic background (Brosseau et al., 2019) (data not shown).

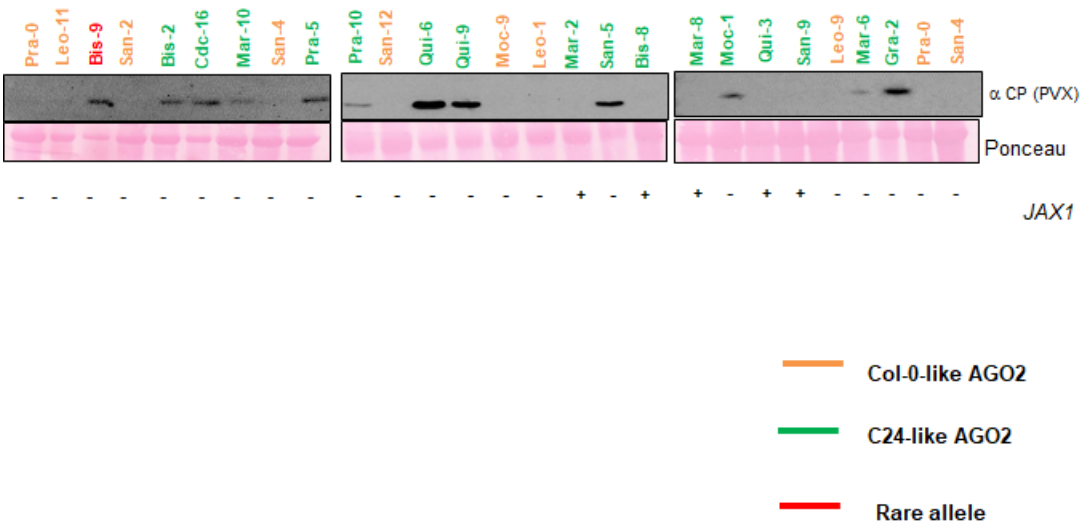

Figure 2.1 Genetic variation in *AGO2* of natural Iberian Peninsula wild accessions determines susceptibility to PVX. Iberian Peninsula wild accessions were inoculated with PVX. At twenty-one dpi, protein was extracted from upper non-inoculated leaf tissues, followed by SDS-PAGE analysis and incubation with anti-PVX CP immune blotting. Wild accession names are colored according to their *AGO2* allele: Col-0-like, orange; C24-like, green; rare allele, red. Ponceau staining (bottom panel) of the same extracts is shown to indicate equal loading. The wild accessions were also assessed for the presence or absence (+/-) of a functional *JAX1* allele. At least five plants per wild accession were tested in each experiment and experiments were repeated three times.

2.4.3 Susceptibility to TuMV is determined by polymorphisms in *AGO2*

Having established that Col-0-like *AGO2* is required for defense against a potexvirus, the same 27 wild accessions were assessed for their susceptibility to TuMV, a potyvirus that infects *A. thaliana* in the wild and is responsible for multiple virus infection in crops (Ivanov et al., 2016). To visualize the degree of susceptibility to TuMV, we used the previously described suppressor-deficient mutant, expressing green fluorescent protein (TuMV-AS9-GFP) (Garcia-Ruiz et al., 2010). At 7

and 21 dpi, no local infection foci were detected in inoculated leaves or upper non-inoculated leaves of Col-0 or in the eight wild accessions that possessed a Col-0 like *AGO2* (Figure 2.2a and Supplementary Figure 2.3). However, all eighteen wild accessions that possess a C24-like *AGO2* allele, as well as the accession with the rare allele, showed accumulation of TuMV-AS9-GFP foci in inoculated leaves and upper non-inoculated leaves to varying degrees (Figure 2.2b, Supplementary Figure 2.2). As expected, the presence of the *JAX1* allele did not affect TuMV infection outcome (Supplementary Fig. 2.1 and 2.2).

While susceptibility to TuMV correlated well with *AGO2* alleles, virus susceptibility could be determined by other elements in the various genetic backgrounds. To study the effects of the two major *AGO2* variant alleles on potyvirus infection we made use of the RILs. Our results showed that no local infection foci were detected in inoculated leaves or in upper non-inoculated leaves in RILs N35, N62 and N66, which have a Col-0 background with introgressed C24 loci near, but not including *AGO2* (Figure 2.2b). However, lines such as N37 and N55, which have a Col-0 background but have the C24 *AGO2* allele, showed accumulation of TuMV-AS9-GFP in inoculated leaves and upper non-inoculated leaves (Figure 2.2b). Conversely, the presence of a Col-0 *AGO2* allele in a line with a majority C24 background (M39) restored resistance to TuMV-AS9-GFP infection (Figure 2.2b). These results indicate that the Col-0 *AGO2* allele is effective against TuMV, whereas the C24 allele is not.

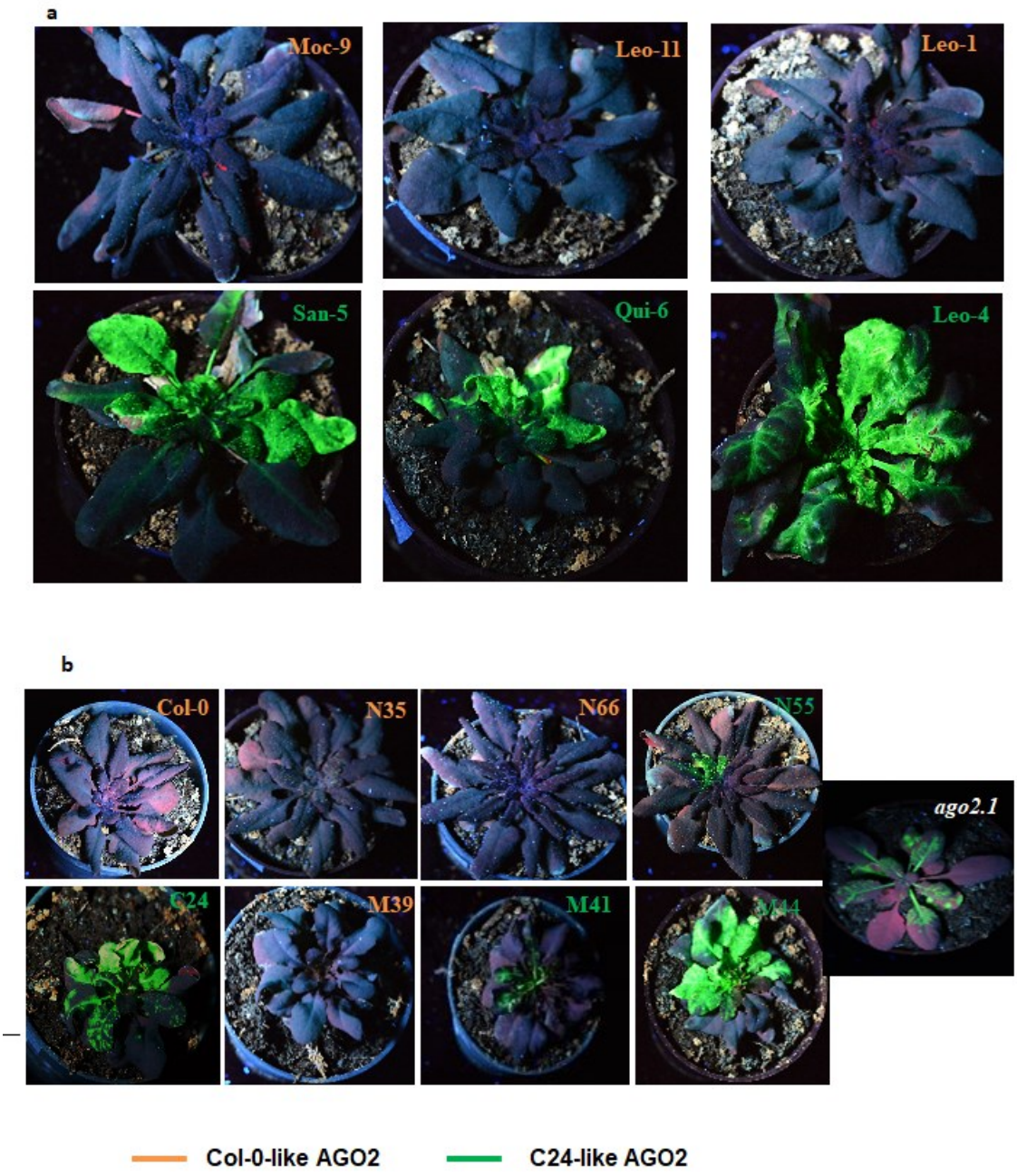

Figure 2.2 Defense against TuMV is determined by *AGO2* allele. *A. thaliana* accessions of different *AGO2* genotypes were inoculated with TuMV-AS9-GFP. a, A subset of Iberian Peninsula wild accessions that possess either a Col-0-like *AGO2* allele (top panel) or a C24-like *AGO2* allele (bottom panel). b, Col-0 and Col-0 background RILs (top panel) and C24 and C24 background RILs (bottom panel). Names of wild accession and RILs are color coded according to their *AGO2* allele identity: Col-0-like, orange, C24-like, green and the *ago2.1* (Col-0 background) mutant is

depicted in white. Plants were photographed under UV illumination at fifteen dpi. Three plants per accession were tested in each experiment and experiments repeated three times.

2.4.4 Resistance to CMV is not determined by *AGO2*

To investigate a possible involvement of *AGO2* in resistance to CMV infection, twenty Iberian Peninsula wild accessions were mechanically inoculated with two strains of CMV, Fny-CMV and Cdc-CMV. As none of the accessions showed immunity or hypersensitivity upon CMV inoculation (Montes et al., 2019), differences in susceptibility to CMV were estimated by quantifying CMV RNA accumulation in upper non-inoculated leaves at fifteen dpi. No association was observed between the identity of *AGO2* allele and accumulation of either strains of CMV in upper non-inoculated tissues. The average viral RNA accumulation of both strains of CMV were similar in accessions encoding a C24-like *AGO2* allele as those encoding Col-0-like *AGO2* alleles (Fny-CMV $Wald\ X^2_{1,87}$=1.280, P=0.257 and Cdc-CMV $Wald\ X^2_{1,91}$=0.053, P=0.814) (Figure 2.3).

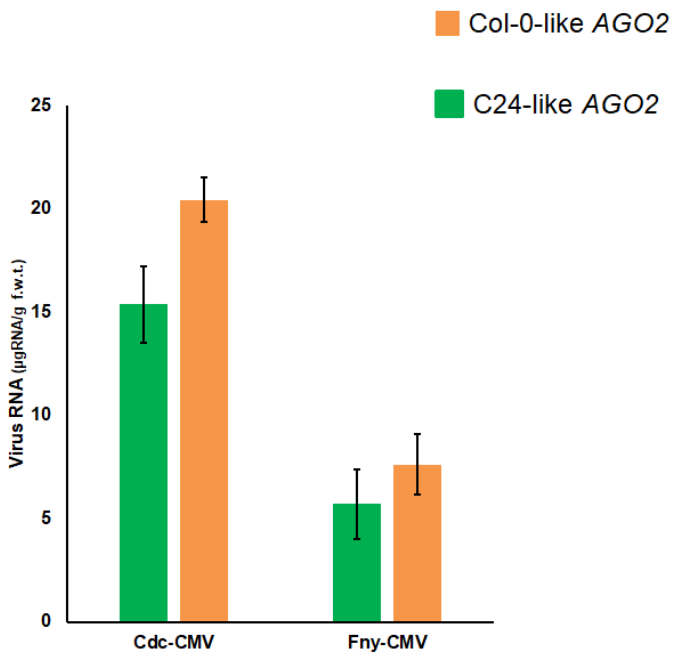

Figure 2.3 Lack of correlation between *AGO2* alleles and accumulation of CMV RNA. Plants of different wild accessions were inoculated at the three-leaf stage and total RNA was harvested

from upper non-inoculated leaves at fifteen dpi. Virus RNA was then quantified by dot-blot hybridization. Data are mean values and error bars represent standard error of at least five experimental replicates with five plants in each replicate. Twenty wild accessions were tested, and accessions are categorized according to their *AGO2* alleles, with C24-like *AGO2* accessions indicated as green bar plots and Col-0-like *AGO2* accessions indicated in orange.

2.4.5 C24-like *AGO2* allele is associated with a reproductive advantage in the absence of virus infection.

The relationship between the resources allocated to vegetative growth and to reproduction can be estimated in *A. thaliana* by examining the rosette weight (*RW*), seed weight *(SW)* and inflorescence weight without seed (*IW),* with RW indicative of vegetative growth and reproductive effort indicated by *SW* and *IW* (Pagán et al., 2009, 2008, 2007). The (*SW+IW)/RW* ratio defines a feature on the basis of which accessions can be clustered into two allometric (Montes et al., 2019; Pagán et al., 2008) (Supplementary Table 2.1). Group 1 accessions have a lower ratio, indicating greater growth of vegetative tissue, associated with a longer life cycle, whereas a higher ratio in group 2 indicates a greater allocation of resources towards reproduction (Pagán et al., 2008). Of the wild accessions studied herein, twelve clustered in group 1 (Supplementary Table 2.1), of which half possessed a C24-like *AGO2*. In contrast, seven of eight accessions clustering in group 2, encode a C24-like *AGO2* (Table 2.2 and Supplementary Table 2.1). Although both types of *AGO2* alleles were present in each allometric group, there was a significant association ($X^2_{1,19}=2.967, P=0.084$) between the nature of the *AGO2* allele and plant allometry.

The *SW* of wild accessions with a C24-like *AGO2* was significantly higher than those with a Col-0-like *AGO2* (Wald $X^2_{1,94}$=4.565, P=0.003*)* (Figure 2.4a and Table 2.2). *IW* was similar between both allele categories (*Wald* $X^2_{1,94}=2.541, P=0.111$). From these analyses, we infer that accessions with a C24-like *AGO2* allele invest more resources in reproduction than those with a Col-0-like *AGO2*.

To reduce confounding effects due to different genetic backgrounds, we also measured *IW, RW* and *SW* in the same set of RILs analyzed for virus resistance. All lines with a C24-like *AGO2*, regardless of genetic background, had a higher *IW* ($F_{1,90}$ = 37.81, P=2.11 X 10^{-8}), and *SW* ($F_{1,90}$ = 13.47, P=7.27 X 10^{-10}) than lines with a Col-0-like *AGO2*. However, there was no difference in *RW* ($F_{1,90}$ = 0.009, P= 0.925) between the two groups of accessions with different *AGO2* alleles (Figure 2.5b). The evaluations of life history traits for the RILs were performed in different growth conditions than the wild accessions (see materials and methods), resulting in differences in absolute weights with the wild accessions (Figure 2.5). Nonetheless, these results validate an association between C24-like *AGO2* alleles and higher seed production in the absence of virus infection (Figures 2.4 a and b).

Table 2.2. Mean values of rosette, inflorescence and seed weight of mock inoculated plants of Iberian *A. thaliana* wild accessions.

	C24-like *AGO2* [a]	Col-0-like *AGO2*[a]
RW[b]	0.42±0.10	0.56±0.10
IW[b]	3.10±0.27	2.99±0.15
SW[b]	1.00±0.06	0.78±0.09

[a]: Mean values and standard error of at least five experimental replicates
[b] Traits are: Rosette weight (*RW*), inflorescence without seed weight (*IW*) and viable seed weight (*SW*), expressed in g.

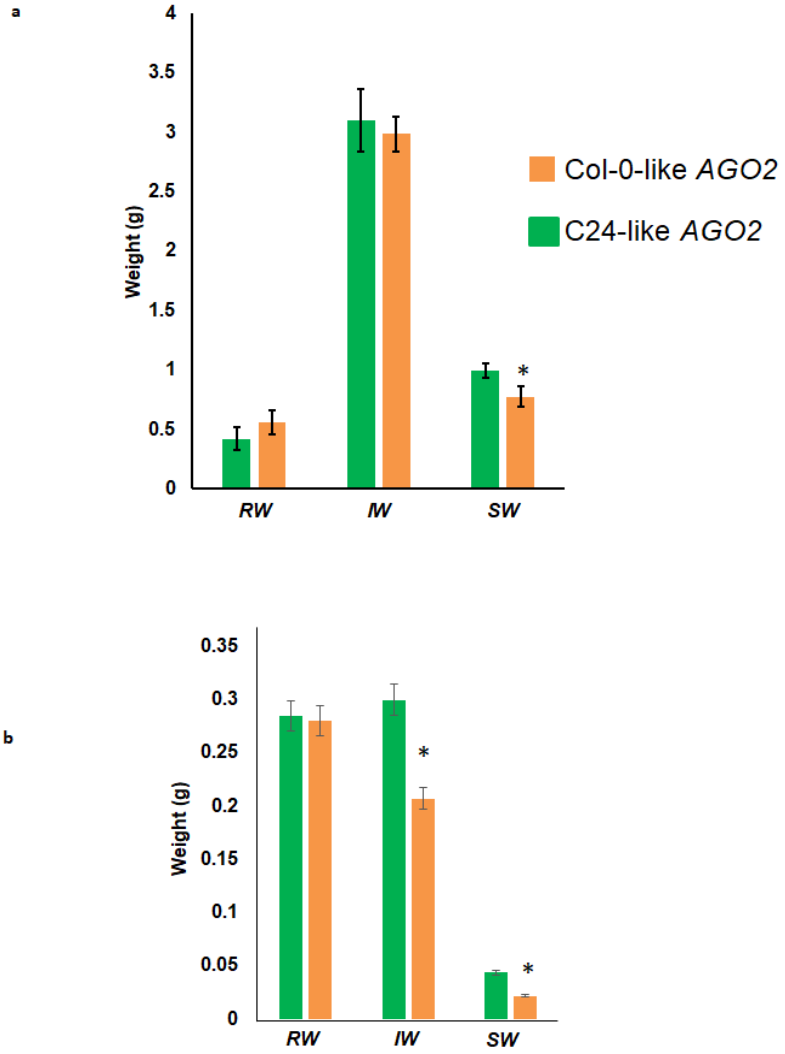

Figure 2.4 Life history analyses of accessions and RILs supports a reproductive advantage for C24-like *AGO2* alleles. a, valuation of rosette weight (*RW*), inflorescence weight without seed (*IW*) and seed weight (*SW*) (g) from mock inoculated Iberian Peninsula wild accessions. Accessions with C24-like *AGO2* alleles are indicated as the green bar plots and those with a Col-0-like *AGO2* indicated as the orange bar plots. Data represent mean values and standard error of five individual plants per wild accession, with five experimental replicates. Asterisks indicate significant differences between groups ($P<0.05$). b, Evaluation of *RW, IW* and *SW* (g) of RILs. Lines with C24-like *AGO2* alleles are indicated as the green bar plots and those with a Col-0-like *AGO2* indicated the orange bar plots. Data are mean values of thirty individual plants per RIL with ±standard error bars. Asterisks indicate significant differences between groups ($P<0.05$).

2.4.6 Col-0 *AGO2* confers a reproductive advantage in the presence of TuMV infection.

To investigate the effect of Col-0-like *AGO2* on plant fitness in the presence of a viral infection, RILs were inoculated with diluted sap containing TuMV-GFP. Symptoms of plants were observed over a forty-five-day period. In C24 and lines with C24 *AGO2* (N37, and M41), strong disease symptoms were apparent, including severe stunting, accelerated leaf senescence, and tissue necrosis, regardless of the genetic background (Figure 2.5a). In contrast, despite initial accumulation of GFP in inoculated plants these symptoms were largely absent in Col-0 and lines with a Col-0-like *AGO2* after forty-five days. Furthermore, although seed set was somewhat reduced compared to uninfected plants, lines expressing Col-0 AGO2 were nonetheless able to produce seed, in contrast to TuMV-infected lines expressing C24 AGO2, which did not (Figure 2.5a). Similar results were seen when the same lines were infected with a VSR-defective mutant, TuMV-AS9-GFP (Figure 2.5b).

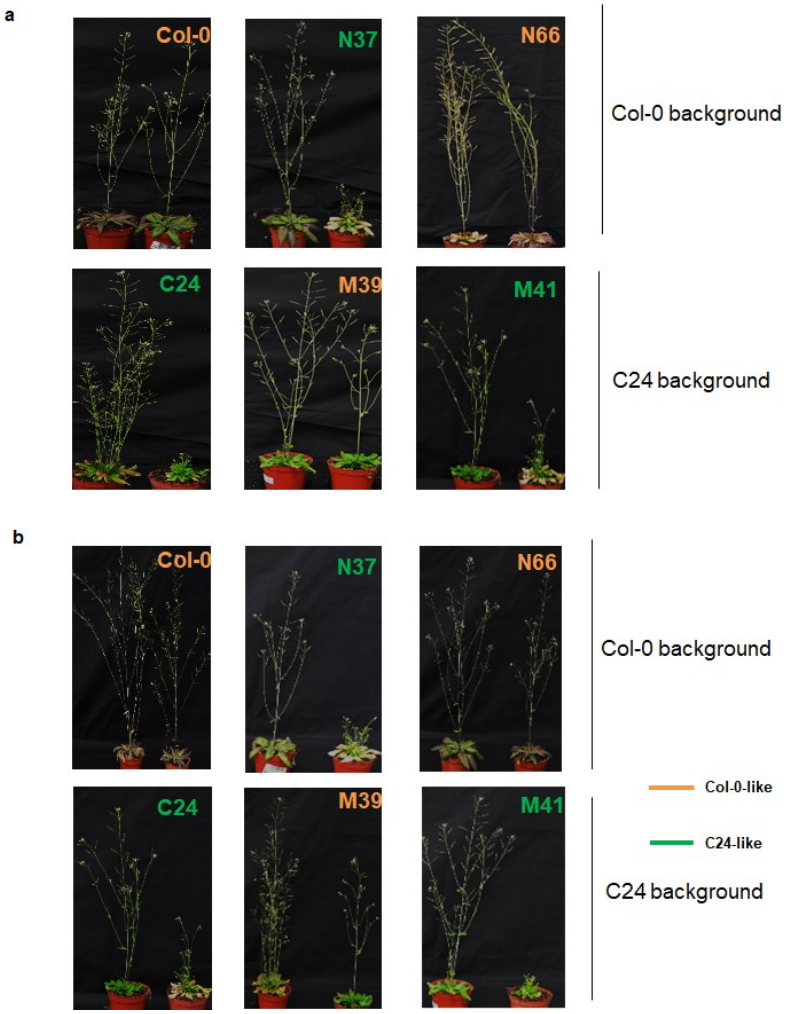

Figure 2.5 Col-0 *AGO2* confers a reproductive advantage in the presence of TuMV. a. A subset of RILs with Col-0 and C24 backgrounds, as indicated, were infected with TuMV-GFP (a) or TuMV-AS9-GFP (b). Plants were photographed at forty-five dpi, showing mock inoculated plants on the left and TuMV-infected plants on the right. RILs are color coded according to their *AGO2* allele identity: Col-0-like, orange, C24-like, green.

2.5 DISCUSSION

Under varying conditions and in the presence or absence of pathogens, plants often make trade-offs between growth, defense and reproduction (for review, see (Chae et al., 2016; Miyashita et al., 2016)). In plants, interactions with viruses appear to have played major roles in affecting life

history traits essential for reproduction and survival in natural populations (Montes et al., 2019; Pagán et al., 2009, 2008; Shukla et al., 2018). Indeed, we have shown that the *AGO2* gene appears to have been subjected to balancing selection to maintain two major types of alleles (Brosseau et al., 2019). We have confirmed and extended this analysis to show that only one of these has effective antiviral activity against PVX. By investigating polymorphisms in the *AGO2* gene in naturally occurring *A. thaliana* wild accessions from the Iberian Peninsula we also show that these polymorphisms are important in the interaction with one virus that naturally infects *A. thaliana* (TuMV), but not another (CMV). Col-0 AGO2 confers a reproductive advantage in the presence of TuMV, whereas the C24-like AGO2 variants confer an advantage in the absence of virus. These observations demonstrate a trade-off between defense and growth strategies and provide an explanation for the prevalence of both alleles.

The co-occurrence of functionally distinct alleles across both global and local populations of *A. thaliana* suggests that *AGO2* is under balancing selection. Balancing selection may be driven by a number of biotic or abiotic factors (Todesco et al., 2010). Our previous analysis of genetically distinct Eurasian accessions showed an approximately 1:1 ratio of C24-like to Col-0-like alleles (Brosseau et al., 2019), whereas the current analysis showed an approximately 2:1 ratio (Figure 2.1). These results raise the question of why apparently non-functional variants of AGO2 have been maintained at such high frequencies. With respect to virus resistance, the C24-like *AGO2* alleles behave similarly to *ago2* null alleles. However, we have previously shown that C24 AGO2 in fact retains most of its molecular functions, including roles in regulating endogenous genes via miRNAs and in DNA methylation (Brosseau et al., 2019). As such, it is possible that the C24-like alleles have been maintained because they provide a gain of function that confers a selective advantage in certain conditions. A gain of function for reproductive success would be consistent with the fact that we do not observe alleles predicted to encode non-functional proteins ((Brosseau et al., 2019); this report) and that Col-0 null mutants (*ago2.1*) show reproductive/vegetative traits similar to WT Col-0 (Supplemental Figure 2.3). The co-existence of wild *A. thaliana* accessions that are susceptible or resistant to CMV infection has been observed in the wild, consistent with trade-offs between reproduction and virus resistance (Pagán et al., 2010a). The identification of different variants of AGO2 affecting fitness in the presence of absence of virus is, to our knowledge, the first description of the genetic basis of such a trade-off.

In addition to the implications for plant defense, our results are also pertinent to studies of virus ecology. In particular, plants with Col-0-like *AGO2*, although susceptible to TuMV infection, appear to be relatively tolerant and may act as reservoirs for different viruses. Our results also highlight the importance of considering AGO2 genotypes in plant-virus studies. That is, many studies are undertaken using the Col-0 accession (Carbonell et al., 2012; Garcia-Ruiz et al., 2015; Jaubert et al., 2011), which may reflect only a portion of natural *A. thaliana* accessions. Indeed, to the best of our knowledge, only viruses found to infect *A. thaliana* in the laboratory have been observed infecting plants in the wild, as these are the only viruses that have been tested for. As such, the virome found in wild *A. thaliana* may be much more diverse than has been reported, including viruses that induce few or no symptoms, as is seen with PVX. Thus, the use of multiple accessions when studying viruses in *A. thaliana* could be beneficial in certain cases.

CMV naturally infects *A. thaliana* in the wild (Pagán et al., 2010a) and AGO2 has been reported as being required for defense against CMV (Harvey et al., 2011; Wang et al., 2011). However, our results indicate that differences in *AGO2* alleles do not appear to have a major influence on CMV infections (Figure 2.4). This suggests that wild-type CMV is likely able to overcome the effects of AGO2 via an effective VSR and/or that other genetic factors affect CMV susceptibility. Indeed, the C24 ecotype has been reported to be resistant to the CMV(Y) strain due to the presence of a single dominant locus of *RCY1* [resistant to CMV(Y)], encoding an NLR protein (Takahashi et al., 2002, 2001). However, RCY1 does not confer resistance to the strains used in this report.

Based on our results, we propose a model where it is advantageous for a plant to possess a C24-like *AGO2* allele in the absence of viral infection and that selection pressure may have resulted in multiple independent appearances of these alleles (Brosseau et al., 2019). We also propose that this is due to a gain-of-function for the C24 *AGO2* alleles, although it is unclear what C24-like AGO2 proteins might do that Col-0-like AGO2 proteins do not. At the same time, Col-0-like *AGO2* alleles are important for reproductive success in the presence of virus. These findings could explain why both alleles co-exist in local natural populations. In conclusion, findings from the current study broadens our understanding of the allelic variations present in the *AGO2* gene and how these variations are essential for defense against PVX and TuMV infections, as well as

reproductive efforts. Although all infections assay and life history analysis were done on plants grown in the controlled environments, results from this study are important in understanding the effects that genetic variation has on pathogen susceptibility in wild plant populations. Future work should investigate what makes *AGO2* important for some virus infection and not for others, the extent of *AGO2* polymorphisms in other plant species, and how changes in environmental factors affect the roles of these *AGO2* alleles.

2.6 MATERIALS AND METHODS

2.6.1 Plant material and growth condition.

For analyses using wild accessions, seeds were stratified (darkness, 4ºC) for seven days before germination at 25/20 ºC day/night, 16 h light. Ten-day-old seedlings were transferred to 4 ºC, 8 h light, for vernalisation for eight weeks, depending on the experiment. After vernalization, plants were transplanted to 0.43 L pots and returned to the greenhouse, where they were kept (25/20 ºC day/night, 16 h light) until the end of the experiment. For all other experiments, *A. thaliana* plants were cultivated using Agromix (Fafard, Saint-Bonaventure, QC) substrate under 12h light/12 hours dark photoperiod at 23 °C. *A. thaliana* seeds were vernalized at 4 ^{0}C before they were transferred to the growth chamber for germination. Col-0 (CS28168) and C24 (CS28127) were obtained from the ABRC stock center. The *ago2-1* mutant in Col-0 background has been described elsewhere (Takeda et al., 2008). Reciprocal Inbred lines (RILs) of Col-0 and C24 backgrounds were provided by R.C. Meyer and have been previously described (Törjék et al., 2008). Briefly, these RILs were generated by crossing Col-0 and C24 plants, backcrossing the progenies to the parental lines and then self-crossing the resulting lines. The results of these crosses are lines with the majority of their genome from one parental line, and a small genomic region from the other (Törjék et al., 2008).

2.6.2 Virus isolates and inoculations.

Two subgroup IA CMV isolates were used, Fny-CMV and Cdc-CMV, which differ in the sequence of their genomic RNAs in about 1% of positions. Fny-CMV is a well-characterized reference isolate (Palukaitis et al., 1992). Cdc-CMV was isolated from field-infected *A. thaliana* plants from the population at Ciruelos de Coca, Spain in 2008 (Montes et al., 2019). Isolates were multiplied in *Nicotiana clevelandii*, Fny-CMV from transcripts of cDNA clones and Cdc-CMV from biological clones derived from local lesions in *Chenopodium quinoa*. *A. thaliana* plants were mechanically inoculated at the five-leaf stage (stage 1.05, (Boyes et al., 2001)) with 15 µl of sap from infected *N. clevelandii* leaves in 0.01M phosphate buffer pH 7.0, 0.2% sodium diethyldithiocarbamate. 15 µl of buffer were applied to mock-inoculated controls. All plants in each experiment were grown in a completely randomized design. For PVX, TuMV-GFP and TuMV-AS9-GFP, virus inoculation was performed at the five-leaf stage. Viral saps were produced with PVX, TuMV-GPP or TuMV-AS9-GFP-infected *N. benthamiana* plant material. Infected material was ground in 0.1 M phosphate buffer, pH 7.0 (2 mL/g of infected tissue). Viral sap was diluted until no visual accumulation of TuMV-AS9-GFP was observed in Col-0, as described in (Garcia-Ruiz et al., 2015, 2010). For TuMV-GFP infection, viral sap was diluted by a factor of 0.25. Mock inoculations were performed with sap produced with uninfected *N. benthamiana* plants (2 mL/g of healthy tissues). Inoculations were carried out by rub inoculating 10µl of viral sap on three leaves per plant in the presences of silicone carbide. Twenty-one days post-inoculation (21 dpi), aerial parts or inflorescences of infected plants were pooled and analyzed. To monitor TuMV-AS9-GPF accumulation, GFP was visualized using a hand-held UV lamp (UVP Blak-Ray-100AP high-intensity) at seven, nine, eleven, fourteen, sixteen, nineteen- and twenty-one-days post inoculation.

2.6.3 Protein extraction and Western Blotting.

For viral accumulation analysis, 1 g of fresh tissue was ground in liquid nitrogen in 1.5 mL of protein extraction buffer (20mM Tris-HCl [pH 7.4], 100mM KCl, 2.5mM $MgCl_2$, 0.1% NP-40, supplemented with 1X protease inhibitor cocktail). Total protein extracts were centrifuged at 16,000 x g for 10 minutes at 4 ^{0}C. For PVX detection, 1.5X of Laemmli loading buffer (100mM {Tris (pH 6.8), 4% (w/v)} SDS, 0.2% bromophenol blue, 20% glycerol, 200mM DTT) was added

to 60µl of the supernatant and heated to 95°C for 10 minutes (Laemmli, 1970). After the extraction, proteins were resolved using SDS-PAGE and detected by immune blotting as previously described in (Brosseau and Moffett, 2015).

2.6.4 Sequencing and Polymerase Chain Reaction.

To genotype *AGO2* alleles, genomic plant DNA was extracted using DNA extraction buffer (50mM NaCl, 100mM tris-HCl pH 8.0, 50mM EDTA pH 8.0) using a phenol-chloroform extraction method. DNA pellets were re-suspended in ddH_2O at a concentration of 20-100ng and then used as template for the PCR reaction. Each PCR reaction contained ThermoPol Buffer 10X, Taq DNA Polymerase (New England Biolabs), 0.2mM of dNTP mix, 1.5mM of $MgCl_2$, 0.6 µM of specific primers either for Col-0 like *AGO2* (Col-0AGO2F: 5'-GTTCAGTAGGAGGAGTCGC-3') or C24-like *AGO2* detection (C24AGO2F: 5'-CGGTTCAGTAGGAGGTGTCGA-3') with a reverse primer sequence of (5'-GCTAAGGGAAACTCATCGGG-3'). PCR mixtures for *AGO2* detection were incubated for 2 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 40 s, and PCR products were analyzed by electrophoresis on 2% agarose gels and PCR products were sequenced with the reverse primer AGO2seq:5'-GGAACCTGTGGCTTACGA-3'. For the identification of *JAX1* gene in the wild accessions, DNA extraction was performed as described above and the following primers were used (JAX1pF:5'-ACTATGTCAAGAATGGTCAA-3') with a reverse primer sequence of (JAX1pR: 5'-GGTAAGGTTTTTATCAAAAG -3'). PCR reagents used are same as described above and PCR procedure and conditions were optimized for this primer set.

2.6.5 Quantification of CMV multiplication.

Virus multiplication in plants was estimated from virus RNA accumulation as described in Pagán et al., (2014). Briefly, at fifteen days post-inoculation 0.01 g fresh weight (fwt) of leaf tissue was harvested from four different upper non-inoculated infected leaves. Nucleic acids were extracted from the pooled leaf tissue using TRI-reagent (Sigma-Aldrich, St Louis, MO, USA). Virus RNA

was then quantified by dot-blot hybridization with ^{32}P-labelled RNA probes complementary to nucleotides 1933–2215 of Fny-CMV RNA3 (GeneBank Acc. No. D10538). In each blot, internal standards for Fny-CMV and Cdc-CMV were included as a two-fold dilution series (1–0.001 μg) of purified virion RNA in nucleic acid extracts from non-inoculated plants. Mock-inoculated samples served as negative controls. Nucleic acid extracts were blotted at different dilutions to ensure that hybridization signal was on the linear portion of the RNA concentration-hybridization curve. As loading controls, parallel membranes were hybridized with a cDNA probe of *β-tubulin chain 2* (*TUB2*) mRNA of *A. thaliana* (1086–1568nt, GeneBank Acc. No. NM_125664.4).

2.6.6 Quantification of life-history traits.

Rosette weight was used to estimate vegetative growth effort, inflorescence plus seed weight to estimate total reproductive effort, and seed weight to estimate progeny production (Thompson and Stewart, 1981). Plants were harvested at complete senescence and dry weight of rosettes (rosette weight, *RW*), inflorescence structures without seeds (inflorescence weight, *IW*) and seeds (seed weight, *SW*) were measured separately (g). For the quantification. Plants were harvested at the complete senescence stage and dry weight was determined after plants were maintained at 55 °C in an oven until constant weight was attained. The weights of rosettes (rosette weight, *RW*), inflorescence structures including seeds (inflorescence weight, *IW*) and seeds (seed weight, *SW*) (g) were measured separately (according to (Pagán et al., 2008). This is the time period that is dedicated to flower production.

2.6.7 Statistical Analysis.

Association between allometry group and *AGO2* alleles were analyzed by Chi-squared test. Differences in *RW, IW*, *SW* and virus accumulation were analyzed by generalized linear models (GLMz) considering *AGO2* genotype and wild accessions nested to *AGO2* genotype, as random factors. Seed weight of the mock-inoculated plants *(SW*) was normally distributed according to Kolmogorov Smirnoff. The other variables were transformed to be normal and in otherwise cases,

were analysed with normal distribution and identity link function (based on the smallest AIC value among a set of alternative models). For the RILs life history trait assessment, normality of data distribution was evaluated by a Shapiro–Wilk test and homoscedasticity and analysis of the different life history traits between groups was done using one-way ANOVA to determine if there was any difference between the t*wo AGO2* alleles for these life history traits. For individual RILs, the statistical analysis of *IW* and *RW* was determined by performing Least Significant Difference (LSD) analyses. All analyses were performed with nortest (Gross Juergen, 2015), rriskDistributions (Belgorodski, 2017), lme4 (Bates et al., 2015) and stat R package using the R version 3.4.4.

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2.8 Contributions

A.A., F.G.A and P.M. conceived and designed the experiments. A.A., N.M. and C.B. performed the experiments. A.A., and N.M. analyzed the data. A.A., N.M, F.G.A. and P.M wrote the manuscript.

2.9 References

Adams, M.J., Antoniw, J.F., Fauquet, C.M., 2005. Molecular criteria for genus and species

discrimination within the family Potyviridae. Arch. Virol. 150, 459–479. https://doi.org/10.1007/s00705-004-0440-6

Adenot, X., Elmayan, T., Lauressergues, D., Boutet, S., Bouché, N., Gasciolli, V., Vaucheret, H., 2006. DRB4-Dependent TAS3 trans-Acting siRNAs Control Leaf Morphology through AGO7. Curr. Biol. 16, 927–932. https://doi.org/10.1016/j.cub.2006.03.035

Agorio, A., Vera, P., 2007. ARGONAUTE4 is required for resistance to Pseudomonas syringae in Arabidopsis. Plant Cell 19, 3778–90. https://doi.org/10.1105/tpc.107.054494

Akbergenov, R., Si-Ammour, A., Blevins, T., Amin, I., Kutter, C., Vanderschuren, H., Zhang, P., Gruissem, W., Meins, F., Hohn, T., Pooggin, M.M., 2006. Molecular characterization of geminivirus-derived small RNAs in different plant species. Nucleic Acids Res. 34, 462–471. https://doi.org/10.1093/nar/gkj447

Alazem, M., He, M.-H., Moffett, P., Lin, N.-S., 2017. Abscisic Acid Induces Resistance against Bamboo Mosaic Virus through Argonaute2 and 3. Plant Physiol. 174, 339–355. https://doi.org/10.1104/pp.16.00015

Anandalakshmi, R., Marathe, R., Ge, X., Herr, J.M., Mau, C., Mallory, A., Pruss, G., Bowman, L., Vance, V.B., 2000. A Calmodulin-Related Protein That Suppresses Posttranscriptional Gene Silencing in Plants. Science (80-.). 290.

Andika, I.B., Maruyama, K., Sun, L., Kondo, H., Tamada, T., Suzuki, N., 2015. Differential contributions of plant Dicer-like proteins to antiviral defences against potato virus X in leaves and roots. Plant J. 81, 781–793. https://doi.org/10.1111/tpj.12770

Ange´lique Deleris, Javier Gallego-Bartolome, Jinsong Bao, Kristin D. Kasschau, James C. Carrington, O.V., 2006. Hierarchical Action and Inhibition of Plant Dicer-Like Proteins in Antiviral Defense. Science (80-.). 305, 1462–322. https://doi.org/10.1126/science.1128650

Axtell, M.J., 2013. Classification and Comparison of Small RNAs from Plants. Annu. Rev. Plant Biol. 64, 137–159. https://doi.org/10.1146/annurev-arplant-050312-120043

Azevedo, J., Garcia, D., Pontier, D., Ohnesorge, S., Yu, A., Garcia, S., Braun, L., Bergdoll, M., Hakimi, M.A., Lagrange, T., Voinnet, O., 2010. Argonaute quenching and global changes in Dicer homeostasis caused by a pathogen-encoded GW repeat protein. Genes Dev. 24,

904–915. https://doi.org/10.1101/gad.1908710

Bakker, E.G., Toomajian, C., Kreitman, M., Bergelson, J., n.d. A Genome-Wide Survey of R Gene Polymorphisms in Arabidopsis W. https://doi.org/10.1105/tpc.106.042614

Bally, J., Nakasugi, K., Jia, F., Jung, H., Ho, S.Y.W., Wong, M., Paul, C.M., Naim, F., Wood, C.C., Crowhurst, R.N., Hellens, R.P., Dale, J.L., Waterhouse, P.M., 2015. The extremophile Nicotiana benthamiana has traded viral defence for early vigour. Nat. Plants 1, 1–6. https://doi.org/10.1038/nplants.2015.165

Balogun, O.S., 2003. Patterns of disease manifestation in tomato seedlings singly or doubly infected with Potato X potexvirus and Tobacco Mosaic tobamovirus.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using **lme4**. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Baumberger, N., Baulcombe, D.C., 2005. Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. Proc. Natl. Acad. Sci. U. S. A. 102, 11928–33.

Bayne, E.H., Rakitina, D. V., Morozov, S.Y., Baulcombe, D.C., 2005. Cell-to-cell movement of Potato Potexvirus X is dependent on suppression of RNA silencing. Plant J. 44, 471–482. https://doi.org/10.1111/j.1365-313X.2005.02539.x

Bejerman, N., Mann, K.S., Dietzgen, R.G., 2016. Alfalfa dwarf cytorhabdovirus P protein is a local and systemic RNA silencing supressor which inhibits programmed RISC activity and prevents transitive amplification of RNA silencing. Virus Res. 224, 19–28. https://doi.org/10.1016/j.virusres.2016.08.008

Belgorodski, N.K.S.K.T., 2017. Package “rriskDistributions” Type Package Title Fitting Distributions to Given Data or Known Quantiles.

Bellaoui, M., Keddie, J.S., Gruissem, W., 2003. DCL is a plant-specific protein required for plastid ribosomal RNA processing and embryo development. Plant Mol. Biol. 53, 531–543.

Bendahmane, A., Kanyuka, K., Baulcombe, D.C., 1999. The Rx Gene from Potato Controls Separate Virus Resistance and Cell Death Responses. Plant Cell 11, 781–791.

Bendahmane, A., Querci, M., Kanyuka, K., Baulcombe, D.C., 2000. Agrobacterium transient expression system as a tool for the isolation of disease resistance genes: application to the Rx2 locus in potato. Plant J. 21, 73–81. https://doi.org/10.1046/j.1365-313x.2000.00654.x

Bengyella, L., Waikhom, S.D., Allie, F., Rey, C., 2015. Virus tolerance and recovery from viral induced-symptoms in plants are associated with transcriptome reprograming. Plant Mol. Biol. 89, 243–252. https://doi.org/10.1007/s11103-015-0362-6

Best, A., White, A., Boots, M., 2008. Maintenance of host variation in tolerance to pathogens and parasites. Proc. Natl. Acad. Sci. U. S. A. 105, 20786–91. https://doi.org/10.1073/pnas.0809558105

Bhattacharjee, S., Zamora, A., Azhar, M.T., Sacco, M.A., Lambert, L.H., Moffett, P., 2009. Virus resistance induced by NB-LRR proteins involves Argonaute4-dependent translational control. Plant J. 58, 940–951. https://doi.org/10.1111/j.1365-313X.2009.03832.x

Blevins, T., Rajeswaran, R., Shivaprasad, P. V., Beknazariants, D., Si-Ammour, A., Park, H.S., Vazquez, F., Robertson, D., Meins, F., Hohn, T., Pooggin, M.M., 2006. Four plant Dicers mediate viral small RNA biogenesis and DNA virus induced silencing. Nucleic Acids Res. 34, 6233–6246.

Bohmert, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M., Benning, C., 1998. AGO1 defines a novel locus of Arabidopsis controlling leaf development. EMBO J. 17, 170–180.

Bologna, N.G., Voinnet, O., 2014. The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis. Annu. Rev. Plant Biol. 65. https://doi.org/10.1146/annurev-arplant-050213-035728

Borevitz, J.O., Hazen, S.P., Michael, T.P., Morris, G.P., Baxter, I.R., Hu, T.T., Chen, H., Werner, J.D., Nordborg, M., Salt, D.E., Kay, S.A., Chory, J., Weigel, D., Jones, J.D.G., Ecker, J.R., 2007. Genome-wide patterns of single-feature polymorphism in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 104, 12057–62. https://doi.org/10.1073/pnas.0705323104

Borges, F., Martienssen, R.A., 2015. The expanding world of small RNAs in plants. Nat. Publ. Gr. 16. https://doi.org/10.1038/nrm4085

Bortolamiol, D., Pazhouhandeh, M., Marrocco, K., Genschik, P., Ronique Ziegler-Graff, V., 2007. Report The Polerovirus F Box Protein P0 Targets ARGONAUTE1 to Suppress RNA Silencing. Curr. Biol. 17, 1615–1621. https://doi.org/10.1016/j.cub.2007.07.061

Boualem, A., Dogimont, C., Bendahmane, A., 2016. The battle for survival between viruses and their host plants. Curr. Opin. Virol. https://doi.org/10.1016/j.coviro.2015.12.001

Bouché, N., Lauressergues, D., Gasciolli, V., Vaucheret, H., 2006. An antagonistic function for Arabidopsis DCL2 in development and a new function for DCL4 in generating viral siRNAs. EMBO J. 25, 3347–3356. https://doi.org/10.1038/sj.emboj.7601217

Boutrot, F., Zipfel, C., 2017. Function, Discovery, and Exploitation of Plant Pattern Recognition Receptors for Broad-Spectrum Disease Resistance. Annu. Rev. Phytopathol. 55, 257–286. https://doi.org/10.1146/annurev-phyto-080614-120106

Boyes, D.C., Zayed, A.M., Ascenzi, R., Mccaskill, A.J., Hoffman, N.E., Davis, K.R., Görlach, J., 2001. Growth Stage-Based Phenotypic Analysis of Arabidopsis: A Model for High Throughput Functional Genomics in Plants, The Plant Cell.

Brodersen, P., Sakvarelidze-Achard, L., Bruun-Rasmussen, M., Dunoyer, P., Yamamoto, Y.Y., Sieburth, L., Voinnet, O., 2008. Widespread Translational Inhibition by Plant miRNAs and siRNAs. Science (80-.). 320, 1185–1190. https://doi.org/10.1126/science.1159151

Brosseau, C., Adurogbangba, A., Roussin-Léveillée, C., Zhao, Z., Biga, S., Moffett, P., 2019. Natural variation in the Arabidopsis AGO2 gene is associated with susceptibility to potato virus X. bioRxiv 746628. https://doi.org/10.1101/746628

Brosseau, C., Moffett, P., 2015. Functional and Genetic Analysis Identify a Role for Arabidopsis ARGONAUTE5 in Antiviral RNA Silencing. Plant Cell 27, 1742–54. https://doi.org/10.1105/tpc.15.00264

Brosseau, C., Oirdi, M. El, Adurogbangba, A., Ma, X., Moffett, P., 2016. Antiviral Defense Involves AGO4 in an Arabidopsis–Potexvirus Interaction. MPMI 29, 878–888. https://doi.org/10.1094/MPMI-09-16-0188-R

Bucher, E., Lohuis, D., van Poppel, P.M.J.A., Geerts-Dimitriadou, C., Goldbach, R., Prins, M., 2006. Multiple virus resistance at a high frequency using a single transgene construct. J.

Gen. Virol. 87, 3697–3701. https://doi.org/10.1099/vir.0.82276-0

Buchmann, R.C., Asad, S., Wolf, J.N., Mohannath, G., Bisaro, D.M., 2009. Geminivirus AL2 and L2 Proteins Suppress Transcriptional Gene Silencing and Cause Genome-Wide Reductions in Cytosine Methylation □. J. Virol. 83, 5005–5013. https://doi.org/10.1128/JVI.01771-08

Cao, J., Schneeberger, K., Ossowski, S., Günther, T., Bender, S., Fitz, J., Koenig, D., Lanz, C., Stegle, O., Lippert, C., Wang, X., Ott, F., Müller, J., Alonso-Blanco, C., Borgwardt, K., Schmid, K.J., Weigel, D., 2011. Whole-genome sequencing of multiple Arabidopsis thaliana populations. Nat. Genet. 43, 956–965. https://doi.org/10.1038/ng.911

Carbonell, A., Carrington, J.C., 2015. Antiviral roles of plant ARGONAUTES. Curr. Opin. Plant Biol. 27, 111–117. https://doi.org/10.1016/j.pbi.2015.06.013

Carbonell, A., Fahlgren, N., Garcia-Ruiz, H., Gilbert, K.B., Montgomery, T.A., Nguyen, T., Cuperus, J.T., Carrington, J.C., 2012. Functional analysis of three Arabidopsis ARGONAUTES using slicer-defective mutants. Plant Cell 24, 3613–29. https://doi.org/10.1105/tpc.112.099945

Carthew, R.W., Sontheimer, E.J., 2009. Origins and Mechanisms of miRNAs and siRNAs. Cell 136, 642–55. https://doi.org/10.1016/j.cell.2009.01.035

Chae, E., Tran, D.T.N., Weigel, D., 2016. Cooperation and Conflict in the Plant Immune System. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1005452

Chellappan, P., Vanitharani, R., Ogbe, F., Fauquet, C.M., 2005. Effect of temperature on geminivirus-induced RNA silencing in plants. Plant Physiol. 138, 1828–41. https://doi.org/10.1104/pp.105.066563

Chen, H., Zhang, L., Yu, K., Wang, A., 2015. Pathogenesis of Soybean mosaic virus in soybean carrying Rsv1 gene is associated with miRNA and siRNA pathways, and breakdown of AGO1 homeostasis. Virology 476, 395–404. https://doi.org/10.1016/j.virol.2014.12.034

Cheng, X., Wang, A., 2017. The Potyvirus Silencing Suppressor Protein VPg Mediates Degradation of SGS3 via Ubiquitination and Autophagy Pathways. J. Virol. 91. https://doi.org/10.1128/jvi.01478-16

Chisholm, S.T., Coaker, G., Day, B., Staskawicz, B.J., 2006. Host-microbe interactions: Shaping the evolution of the plant immune response. Cell.

Chiu, M.-H., Chen, I.-H., Baulcombe, D.C., Tsai, C.-H., 2010. The silencing suppressor P25 of Potato virus X interacts with Argonaute1 and mediates its degradation through the proteasome pathway. Mol. Plant Pathol. 11, 641–9. https://doi.org/10.1111/j.1364-3703.2010.00634.x

Cho, Y.B., Jones, S.I., Vodkin, L.O., 2017. Mutations in Argonaute5 Illuminate Epistatic Interactions of the K1 and I Loci Leading to Saddle Seed Color Patterns in Glycine max. Plant Cell 29, tpc.00162.2017. https://doi.org/10.1105/tpc.17.00162

Clark, R.M., Schweikert, G., Toomajian, C., Ossowski, S., Zeller, G., Shinn, P., Warthmann, N., Hu, T.T., Fu, G., Hinds, D.A., Chen, H., Frazer, K.A., Huson, D.H., Scholkopf, B., Nordborg, M., Ratsch, G., Ecker, J.R., Weigel, D., 2007. Common Sequence Polymorphisms Shaping Genetic Diversity in Arabidopsis thaliana. Science (80-.). 317, 338–342. https://doi.org/10.1126/science.1138632

Cook, D.E., Mesarich, C.H., Thomma, B.P.H.J., 2015. Understanding Plant Immunity as a Surveillance System to Detect Invasion. Annu. Rev. Phytopathol 53, 541–63. https://doi.org/10.1146/annurev-phyto-080614-120114

Cooley, M.B., Pathirana, S., Wu, H.J., Kachroo, P., Klessig, D.F., 2000. Members of the Arabidopsis HRT/RPP8 family of resistance genes confer resistance to both viral and oomycete pathogens. Plant Cell 12, 663–76. https://doi.org/10.1105/tpc.12.5.663

Cosson, P., Schurdi-Levraud, V., Le, Q.H., Sicard, O., Caballero, M., Roux, F., Le Gall, O., Candresse, T., Revers, F., 2012. The RTM resistance to potyviruses in Arabidopsis thaliana: natural variation of the RTM genes and evidence for the implication of additional genes. PLoS One 7, e39169. https://doi.org/10.1371/journal.pone.0039169

Csorba, T., Kontra, L., Burgyán, J., 2015. Viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. Virology. https://doi.org/10.1016/j.virol.2015.02.028

Csorba, T., Lózsa, R., Hutvágner, G., Burgyán, J., 2010. Polerovirus protein P0 prevents the assembly of small RNA-containing RISC complexes and leads to degradation of

ARGONAUTE1. Plant J. 62, 463–472. https://doi.org/10.1111/j.1365-313X.2010.04163.x

Curtin, S.J., Michno, J.-M., Campbell, B.W., Gil-Humanes, J., Mathioni, S.M., Hammond, R., Gutierrez-Gonzalez, J.J., Donohue, R.C., Kantar, M.B., Eamens, A.L., Meyers, B.C., Voytas, D.F., Stupar, R.M., 2016. MicroRNA Maturation and MicroRNA Target Gene Expression Regulation Are Severely Disrupted in Soybean dicer-like1 Double Mutants. Genes, Genomes, Genet. 6, 423–433.

Dempsey, D.A., Klessig, D.F., 2012. SOS - too many signals for systemic acquired resistance? Trends Plant Sci.

Dennis Gonsalves, 2004. Transgenic Papaya in Hawaii and Beyond.

Derrien, B., Baumberger, N., Schepetilnikov, M., Viotti, C., De Cillia, J., Ziegler-Graff, V., Isono, E., Schumacher, K., Genschik, P., 2012. Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. Proc. Natl. Acad. Sci. 109, 15942–15946. https://doi.org/10.1073/pnas.1209487109

Di Serio, F., Schö, H., Iglesias, A., Tarina, C., Bouldoires, E., Meins, F., Phillips, R.L., 2001. Sense-and antisense-mediated gene silencing in tobacco is inhibited by the same viral suppressors and is associated with accumulation of small RNAs. PNAS 98, 6506–6510.

Diao, P., Zhang, Q., Sun, H., Ma, W., Cao, A., Yu, R., Wang, J., Niu, Y., Wuriyanghan, H., Diao, P., Zhang, Q., Sun, H., Ma, W., Cao, A., Yu, R., Wang, J., Niu, Y., Wuriyanghan, H., 2019. miR403a and SA Are Involved in NbAGO2 Mediated Antiviral Defenses Against TMV Infection in Nicotiana benthamiana. Genes (Basel). 10, 526. https://doi.org/10.3390/genes10070526

Dinesh-Kumar, S.P., Tham, W.-H., Baker, B.J., Gajiwala, P., Pasion, R., Whalen, M.C., Stall, R.E., Staskawicz, B.J., 2000. Structure-function analysis of the tobacco mosaic virus resistance gene N. Proc. Natl. Acad. Sci. 97, 14789–14794. https://doi.org/10.1073/pnas.97.26.14789

Donaire, L., Wang, Y., Gonzalez-Ibeas, D., Mayer, K.F., Aranda, M.A., Llave, C., 2009. Deep-sequencing of plant viral small RNAs reveals effective and widespread targeting of viral genomes. Virology 392, 203–214. https://doi.org/10.1016/j.virol.2009.07.005

Duan, C.-G., Fang, Y.-Y., Zhou, B.-J., Zhao, J.-H., Hou, W.-N., Zhu, H., Ding, S.-W., Guo, H.-S., 2012. Suppression of Arabidopsis ARGONAUTE1-Mediated Slicing, Transgene-Induced RNA Silencing, and DNA Methylation by Distinct Domains of the Cucumber mosaic virus 2b Protein. Plant Cell. https://doi.org/10.1105/tpc.111.092718

Duan, C.-G., Zhang, H., Tang, K., Zhu, X., Qian, W., Hou, Y.-J., Wang, B., Lang, Z., Zhao, Y., Wang, X., Wang, P., Zhou, J., Liang, G., Liu, N., Wang, C., Zhu, J.-K., 2015. Specific but interdependent functions for Arabidopsis AGO4 and AGO6 in RNA-directed DNA methylation. EMBO J. 34, 581–592. https://doi.org/10.15252/embj

Dunoyer, P., Schott, G., Himber, C., Meyer, D., Takeda, A., Carrington, J.C., Voinnet, O., 2010. Small RNA Duplexes Function as Mobile Silencing Signals Between Plant Cells. Science (80-.). 328.

Dzianott, A., Sztuba-Solińska, J., Bujarski, J.J., 2012a. Mutations in the Antiviral RNAi Defense Pathway Modify Brome mosaic virus RNA Recombinant Profiles. Mol. Plant-Microbe Interact. 25, 97–106. https://doi.org/10.1094/MPMI-05-11-0137

Dzianott, A., Sztuba-Solińska, J., Bujarski, J.J., 2012b. Mutations in the Antiviral RNAi Defense Pathway Modify Brome mosaic virus RNA Recombinant Profiles 25, 97–106.

Eamens, A., Wang, M.-B., Smith, N.A., Waterhouse, P.M., 2008. RNA silencing in plants: yesterday, today, and tomorrow. Plant Physiol. 147, 456–68. https://doi.org/10.1104/pp.108.117275

El-Shami, M., Pontier, D., Lahmy, S., Braun, L., Picart, C., Vega, D., Hakimi, M.A., Jacobsen, S.E., Cooke, R., Lagrange, T., 2007. Reiterated WG/GW motifs form functionally and evolutionarily conserved ARGONAUTE-binding platforms in RNAi-related components. Genes Dev. 21, 2539–2544. https://doi.org/10.1101/gad.451207

El Oirdi, M., El Rahman, T.A., Rigano, L., El Hadrami, A., Rodriguez, M.C., Daayf, F., Vojnov, A., Bouarab, K., 2011. *Botrytis cinerea* Manipulates the Antagonistic Effects between Immune Pathways to Promote Disease Development in Tomato. Plant Cell 23, 2405–2421. https://doi.org/10.1105/tpc.111.083394

Elsharkawy, M.M., Shimizu, M., Takahashi, H., Ozaki, K., Hyakumachi, M., 2013. Induction of

Systemic Resistance against Cucumber mosaic virus in Arabidopsis thaliana by Trichoderma asperellum SKT-1. Plant Pathol. J. 29, 193. https://doi.org/10.5423/PPJ.SI.07.2012.01

Enard, D., Cai, L., Gwennap, C., Petrov, D.A., 2016. Viruses are a dominant driver of protein adaptation in mammals. Elife 5, 1–25. https://doi.org/10.7554/eLife.12469

Eun, C., Lorkovic, Z.J., Naumann, U., Long, Q., Havecker, E.R., Simon, S.A., Meyers, B.C., Matzke, A.J.M., Matzke, M., Freitag, M., 2011. AGO6 Functions in RNA-Mediated Transcriptional Gene Silencing in Shoot and Root Meristems in Arabidopsis thaliana. https://doi.org/10.1371/journal.pone.0025730

Fagard, M., Boutet, S., Morel, J.-B., Bellini, C., Vaucheret, H., 2000. AGO1, QDE-2, and RDE-1 are required for post-transcriptional gene silencing in plants , quelling in fungi , and RNA interference in animals. Proc. Natl. Acad. Sci. 97, 11650–11654.

Fang, X., Qi, Y., 2016. RNAi in Plants: An Argonaute-Centered View. Plant Cell 28, 272–85. https://doi.org/10.1105/tpc.15.00920

Fang, Y.-Y., Zhao, J.-H., Liu, S.-W., Wang, S., Duan, C.-G., Guo, H.-S., 2016. CMV2b-AGO Interaction Is Required for the Suppression of RDR-Dependent Antiviral Silencing in Arabidopsis. Front. Microbiol. 7, 1329. https://doi.org/10.3389/fmicb.2016.01329

Fátyol, K., Ludman, M., Burgyán, J., P.D., Z., V., S., T., T., D.J., P., H., V., W.M., K., R., C., S., C., G.J., H., Y., Q., 2016. Functional dissection of a plant Argonaute. Nucleic Acids Res. 44, 1384–1397. https://doi.org/10.1093/nar/gkv1371

Feng, L., Duan, C.-G., Guo, H.-S., 2013. Inhibition of *in vivo* Slicer activity of Argonaute protein 1 by the viral 2b protein independent of its dsRNA-binding function. Mol. Plant Pathol. 14, 617–622. https://doi.org/10.1111/mpp.12033

Ferreira, S.A., Pitz, K.Y., 2002. Virus Coat Protein Transgenic Papaya Provides Practical Control of Papaya ringspot virus in Hawaii.

Frost, K.E., Groves, R.L., Charkowski, A.O., 2013. Integrated Control of Potato Pathogens Through Seed Potato Certification and Provision of Clean Seed Potatoes. https://doi.org/10.1094/PDIS-05-13-0477-FE

Fusaro, A.F., Matthew, L., Smith, N.A., Curtin, S.J., Dedic-Hagan, J., Ellacott, G.A., Watson, J.M., Wang, M.-B., Brosnan, C., Carroll, B.J., Waterhouse, P.M., 2006. RNA interference-inducing hairpin RNAs in plants act through the viral defence pathway. EMBO Rep. 7, 1168–75. https://doi.org/10.1038/sj.embor.7400837

Garcia-Ruiz, H., Carbonell, A., Hoyer, J.S., Fahlgren, N., Gilbert, K.B., Takeda, A., Giampetruzzi, A., Ruiz, M.T.G., Mcginn, M.G., Lowery, N., Martinez Baladejo, M.T., Carrington, J.C., -Ruiz, G., Carbonell, H., Js, H., Ding, B., 2015. Roles and Programming of Arabidopsis ARGONAUTE Proteins during Turnip Mosaic Virus Infection. PLoS Pathog 11. https://doi.org/10.1371/journal.ppat.1004755

Garcia-Ruiz, H., Takeda, A., Chapman, E.J., Sullivan, C.M., Fahlgren, N., Brempelis, K.J., Carrington, J.C., 2010. Arabidopsis RNA-Dependent RNA Polymerases and Dicer-Like Proteins in Antiviral Defense and Small Interfering RNA Biogenesis during Turnip Mosaic Virus Infection. Plant Cell 22, 481–496. https://doi.org/10.1105/tpc.109.073056

Gargouri-Bouzid, R., Jaoua, L., Rouis, S., Saídi, M.N., Bouaziz, D., Ellouz, R., 2006. PVY-Resistant Transgenic Potato Plants Expressing an Anti-NIa Protein scFv Antibody. Mol. Biotechnol. 33, 133–140. https://doi.org/10.1385/MB:33:2:133

Ghoshal, B., Sanfaçon, H., 2014. Temperature-dependent symptom recovery in Nicotiana benthamiana plants infected with tomato ringspot virus is associated with reduced translation of viral RNA2 and requires ARGONAUTE 1. Virology 456–457, 188–197. https://doi.org/10.1016/J.VIROL.2014.03.026

Gill, U.S., Lee, S., Mysore, K.S., 2015. Host Versus Nonhost Resistance: Distinct Wars with Similar Arsenals. Phytopathology. https://doi.org/10.1094/phyto-11-14-0298-rvw

Giner, A., Lakatos, L., García-Chapa, M., López-Moya, J.J., Burgyán, J., 2010. Viral Protein Inhibits RISC Activity by Argonaute Binding through Conserved WG/GW Motifs. PLoS Pathog. 6, e1000996. https://doi.org/10.1371/journal.ppat.1000996

Gonzalez-Gaitan, M., Jessell, T., Johnston, L., Laufer, E., McCabe, B., Struhl, G., Deleris, A., Gallego-Bartolome, J., Bao, J., Kasschau, K.D., Carrington, J.C., Voinnet, O., 2004. Hierarchical Action and Inhibition of Plant Dicer-Like Proteins in Antiviral Defense.

Science (80-.). 305, 1462–322. https://doi.org/10.1126/science.1128650

González, I., Martínez, L., Rakitina, D. V., Lewsey, M.G., Atencio, F.A., Llave, C., Kalinina, N.O., Carr, J.P., Palukaitis, P., Canto, T., 2010. Cucumber mosaic virus 2b protein subcellular targets and interactions: Their significance to RNA silencing suppressor activity. Mol. Plant-Microbe Interact. 23, 294–303. https://doi.org/10.1094/MPMI-23-3-0294

González, I., Rakitina, D., Semashko, M., Taliansky, M., Praveen, S., Palukaitis, P., Carr, J.P., Kalinina, N., Canto, T., 2012. RNA binding is more critical to the suppression of silencing function of Cucumber mosaic virus 2b protein than nuclear localization. RNA 18, 771–782. https://doi.org/10.1261/rna.031260.111

Goto, K., Kobori, T., Kosaka, Y., Natsuaki, T., Masuta, C., 2007. Characterization of Silencing Suppressor 2b of Cucumber Mosaic Virus Based on Examination of its Small RNA-Binding Abilities. Plant Cell Physiol. 48, 1050–1060. https://doi.org/10.1093/pcp/pcm074

Gross Juergen, L.U., 2015. Tests for Normality [R package nortest version 1.0-4].

Ha, M., Kim, V.N., 2014. Regulation of microRNA biogenesis. Nat. Rev. Mol. Cell Biol. 15, 509–524.

Haas, G., Azevedo, J., Moissiard, G., Le Geldreich, A., Himber, C., Bureau, M., Fukuhara, T., Keller, M., Voinnet, O., 2008. Nuclear import of CaMV P6 is required for infection and suppression of the RNA silencing factor DRB4. EMBO J. 27129, 2102–2112.

Hamera, S., Song, X., Su, L., Chen, X., Fang, R., 2012. Cucumber mosaic virus suppressor 2b binds to AGO4-related small RNAs and impairs AGO4 activities. Plant J. 69, 104–115.

Harvey, J.J.W., Lewsey, M.G., Patel, K., Westwood, J., Heimstädt, S., Carr, J.P., Baulcombe, D.C., 2011. An antiviral defense role of AGO2 in plants. PLoS One 6.

Hashimoto, M., Neriya, Y., Keima, T., Iwabuchi, N., Koinuma, H., Hagiwara-Komoda, Y., Ishikawa, K., Himeno, M., Maejima, K., Yamaji, Y., Namba, S., 2016. EXA1, a GYF domain protein, is responsible for loss-of-susceptibility to plantago asiatica mosaic virus in *Arabidopsis thaliana*. Plant J. 88, 120–131. https://doi.org/10.1111/tpj.13265

Hauptmann, J., Schraivogel, D., Bruckmann, A., Manickavel, S., Jakob, L., Eichner, N., Pfaff, J.,

Urban, M., Sprunck, S., Hafner, M., Tuschl, T., Deutzmann, R., Meister, G., 2015. Biochemical isolation of Argonaute protein complexes by Ago-APP. Proc. Natl. Acad. Sci. U. S. A. 112, 11841–5. https://doi.org/10.1073/pnas.1506116112

Havecker, E.R., Wallbridge, L.M., Hardcastle, T.J., Bush, M.S., Kelly, K.A., Dunn, R.M., Schwach, F., Doonan, J.H., Baulcombe, D.C., 2010. The Arabidopsis RNA-directed DNA methylation argonautes functionally diverge based on their expression and interaction with target loci. Plant Cell 22, 321–34. https://doi.org/10.1105/tpc.109.072199

Hernández-Lagana, E., Rodríguez-Leal, D., Lúa, J., Vielle-Calzada, J.P., 2016. A multigenic network of ARGONAUTE4 clade members controls early megaspore formation in arabidopsis. Genetics 204, 1045–1056. https://doi.org/10.1534/genetics.116.188151

Hily, J.M., García, A., Moreno, A., Plaza, M., Wilkinson, M.D., Fereres, A., Fraile, A., García-Arenal, F., 2014. The Relationship between Host Lifespan and Pathogen Reservoir Potential: An Analysis in the System Arabidopsis thaliana-Cucumber mosaic virus. PLoS Pathog. 10, e1004492. https://doi.org/10.1371/journal.ppat.1004492

Hohn, T., Vazquez, F., 2011. RNA silencing pathways of plants: Silencing and its suppression by plant DNA viruses ☆. BBA - Gene Regul. Mech. 1809, 588–600. https://doi.org/10.1016/j.bbagrm.2011.06.002

Howell, M.D., Fahlgren, N., Chapman, E.J., Cumbie, J.S., Sullivan, C.M., Givan, S.A., Kasschau, K.D., Carrington, J.C., 2007. Genome-wide analysis of the RNA-DEPENDENT RNA POLYMERASE6/DICER-LIKE4 pathway in Arabidopsis reveals dependency on miRNA- and tasiRNA-directed targeting. Plant Cell 19, 926–42. https://doi.org/10.1105/tpc.107.050062

Hu, P., Zhao, H., Zhu, P., Xiao, Y., Miao, W., Wang, Y., Jin, H., 2019. Dual regulation of Arabidopsis AGO2 by arginine methylation. Nat. Commun. 10, 844. https://doi.org/10.1038/s41467-019-08787-w

Hunter, C., 2006. Trans-acting siRNA-mediated repression of ETTIN and ARF4 regulates heteroblasty in Arabidopsis. Development 133, 2973–2981. https://doi.org/10.1242/dev.02491

Inoue-Nagata, A.K., Lima, M.F., Gilbertson, R.L., Inoue-Nagata, A.K., Lima, M.F., Gilbertson, R.L., 2016. A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. Hortic. Bras. 34, 8–18. https://doi.org/10.1590/S0102-053620160000100002

Ivanov, K.I., Eskelin, K., Bašić, M., De, S., Lõhmus, A., Varjosalo, M., Mäkinen, K., 2016a. Molecular insights into the function of the viral RNA silencing suppressor HCPro. Plant J. 85, 30–45. https://doi.org/10.1111/tpj.13088

Ivanov, K.I., Eskelin, K., Bašić, M., De, S., Lõhmus, A., Varjosalo, M., Mäkinen, K., 2016b. Molecular insights into the function of the viral RNA silencing suppressor HCPro. Plant J. 85, 30–45. https://doi.org/10.1111/tpj.13088

Ivanov, K.I., Eskelin, K., Lõ, A., Mä Kinen Correspondence, K., Mä, K., 2014. Molecular and cellular mechanisms underlying potyvirus infection. J. Gen. Virol. 95, 1415–1429. https://doi.org/10.1099/vir.0.064220-0

Iwasaki, S., Kobayashi, M., Yoda, M., Sakaguchi, Y., Katsuma, S., Suzuki, T., Tomari, Y., 2010. Hsc70/Hsp90 Chaperone Machinery Mediates ATP-Dependent RISC Loading of Small RNA Duplexes. Mol. Cell 39, 292–299. https://doi.org/10.1016/j.molcel.2010.05.015

Jacquemond, M., 2012. Cucumber Mosaic Virus. Adv. Virus Res. 84, 439–504. https://doi.org/10.1016/B978-0-12-394314-9.00013-0

Jain, R.K., Pappu, H.R., Pappu, S.S., Varma, A., Ram, R.D., 1998. Molecular characterisation of papaya ringspot potyvirus isolates from India. Ann. Appl. Biol. 132, 413–425. https://doi.org/10.1111/j.1744-7348.1998.tb05218.x

Jamous, R.M., Boonrod, K., Fuellgrabe, M.W., Ali-Shtayeh, M.S., Krczal, G., Wassenegger, M., 2011. The helper component-proteinase of the Zucchini yellow mosaic virus inhibits the Hua Enhancer 1 methyltransferase activity in vitro. J. Gen. Virol. 92, 2222–2226.

Jaubert, M., Bhattacharjee, S., Mello, A.F.S., Perry, K.L., Moffett, P., 2011. ARGONAUTE2 Mediates RNA-Silencing Antiviral Defenses against Potato virus X in Arabidopsis 1[W][OA]. Plant Physiol. 156. https://doi.org/10.1104/pp.111.178012

Jenner, C.E., Wang, X., Ponz, F., Walsh, J.A., 2002. A fitness cost for Turnip mosaic virus to

overcome host resistance. Virus Res. 86, 1–6. https://doi.org/10.1016/S0168-1702(02)00031-X

Jens Tilsner, O.L., Louveaux, M., Ian M. Roberts, Sean N. Chapman, and Karl J. Oparka, 2013. Replication and trafficking of a plant virus are coupled at the entrances of plasmodesmata. J. Cell Biol 201, 981–995. https://doi.org/10.1083/jcb.201304003

Jones, J.D.G., Dangl, J.L., 2006. The plant immune system. Nature 444, 323–329.

Jones, J.D.G., Vance, R.E., Dangl, J.L., 2016. Intracellular innate immune surveillance devices in plants and animals. Science (80-.). 354, 1117. https://doi.org/10.1126/science.aaf6395

Jouannet, V., Moreno, A.B., Elmayan, T., Vaucheret, H., Crespi, M.D., Maizel, A., 2012. Cytoplasmic Arabidopsis AGO7 accumulates in membrane-associated siRNA bodies and is required for ta-siRNA biogenesis. EMBO J. 31, 1704–1713. https://doi.org/10.1038/emboj.2012.20

Kapoor, M., Arora, R., Lama, T., Nijhawan, A., Khurana, J.P., Tyagi, A.K., Kapoor, S., 2008. Genome-wide identification, organization and phylogenetic analysis of Dicer-like, Argonaute and RNA-dependent RNA Polymerase gene families and their expression analysis during reproductive development and stress in rice. BMC Genomics 9. https://doi.org/10.1186/1471-2164-9-451

Kasschau, K.D., Carrington, J.C., 1998. A counterdefensive strategy of plant viruses: Suppression of posttranscriptional gene silencing. Cell 95, 461–470. https://doi.org/10.1016/S0092-8674(00)81614-1

Keima, T., Hagiwara-Komoda, Y., Hashimoto, M., Neriya, Y., Koinuma, H., Iwabuchi, N., Nishida, S., Yamaji, Y., Namba, S., 2017. Deficiency of the eIF4E isoform nCBP limits the cell-to-cell movement of a plant virus encoding triple-gene-block proteins in Arabidopsis thaliana. Sci. Rep. 7, 1–13. https://doi.org/10.1038/srep39678

Kidner, C.A., Martienssen, R.A., 2005. The role of ARGONAUTE1 (AGO1) in meristem formation and identity. Dev. Biol. 280, 504–517. https://doi.org/10.1016/J.YDBIO.2005.01.031

Kim, V.N., 2005. Small RNAs: classification, biogenesis, and function. Mol. Cells 19, 1–15.

https://doi.org/806 [pii]

Kirino, Y., Kim, N., de Planell-Saguer, M., Khandros, E., Chiorean, S., Klein, P.S., Rigoutsos, I., Jongens, T.A., Mourelatos, Z., 2009. Arginine methylation of Piwi proteins catalysed by dPRMT5 is required for Ago3 and Aub stability. Nat. Cell Biol. 11, 652–658. https://doi.org/10.1038/ncb1872

Kirino, Y., Vourekas, A., Sayed, N., de Lima Alves, F., Thomson, T., Lasko, P., Rappsilber, J., Jongens, T.A., Mourelatos, Z., 2010. Arginine methylation of Aubergine mediates Tudor binding and germ plasm localization. Rna 16, 70–78. https://doi.org/10.1261/rna.1869710

Koenig, D., Jimenez-Gomez, J.M., Kimura, S., Fulop, D., Chitwood, D.H., Headland, L.R., Kumar, R., Covington, M.F., Devisetty, U.K., Tat, A. V., Tohge, T., Bolger, A., Schneeberger, K., Ossowski, S., Lanz, C., Xiong, G., Taylor-Teeples, M., Brady, S.M., Pauly, M., Weigel, D., Usadel, B., Fernie, A.R., Peng, J., Sinha, N.R., Maloof, J.N., 2013. Comparative transcriptomics reveals patterns of selection in domesticated and wild tomato, Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.1309606110

Kontra, L., Csorba, T., Tavazza, M., Lucioli, A., Tavazza, R., Moxon, S., Tisza, V., Medzihradszky, A., Turina, M., Burgyán, J., 2016. Distinct Effects of p19 RNA Silencing Suppressor on Small RNA Mediated Pathways in Plants. PLoS Pathog. 12, e1005935. https://doi.org/10.1371/journal.ppat.1005935

Koornneef, M., Alonso-Blanco, C., Vreugdenhil, D., 2004. NATURALLY OCCURRING GENETIC VARIATION IN *ARABIDOPSIS THALIANA*. Annu. Rev. Plant Biol. 55, 141–172. https://doi.org/10.1146/annurev.arplant.55.031903.141605

Kosakovsky Pond, S.L., Frost, S.D.W., 2005. Not So Different After All: A Comparison of Methods for Detecting Amino Acid Sites Under Selection. Mol. Biol. Evol. 22, 1208–1222. https://doi.org/10.1093/molbev/msi105

Kurihara, Y., Watanabe, Y., 2004. Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. PNAS 101, 12753–12758.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of

bacteriophage T4. Nature 227, 680–685. https://doi.org/10.1038/227680a0

Lakatos, L., Szittya, G., Silhavy, D., Burgyán, J., 2004. Molecular mechanism of RNA silencing suppression mediated by p19 protein of tombusviruses. EMBO J. 23, 876–884.

Lario, L.D., Ramirez-Parra, E., Gutierrez, C., Spampinato, C.P., Casati, P., 2013. ANTI-SILENCING FUNCTION1 Proteins Are Involved in Ultraviolet-Induced DNA Damage Repair and Are Cell Cycle Regulated by E2F Transcription Factors in Arabidopsis 1[C][W][OA]. Plant Physiol. 162, 1164–1177. https://doi.org/10.1104/pp.112.212837

Lau, W., Fischbach, M.A., Osbourn, A., Sattely, E.S., 2014. Key applications of plant metabolic engineering. PLoS Biol. 12, e1001879. https://doi.org/10.1371/journal.pbio.1001879

Laufs, J., Trautt, W., Heyraudt, F., Matzeitt, V., Rogersf, S.G., Schellt, J., Gronenborn, B., 1995. In vitro cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. Biochemistry 92, 3879–3883.

Laura R. Newburn, K.A.W., 2015. Cis-acting RNA elements in positive-strand RNA plant virus genomes. Virology 479, 434–443.

Lee, S.-C., Wu, C.-H., Wang, C.-W., 2010. Traffic of a Viral Movement Protein Complex to the Highly Curved Tubules of the Cortical Endoplasmic Reticulum. Traffic 11, 912–930. https://doi.org/10.1111/j.1600-0854.2010.01064.x

Lee, S., Rojas, C.M., Ishiga, Y., Pandey, S., Mysore, K.S., 2013. Arabidopsis heterotrimeric G-proteins play a critical role in host and nonhost resistance against Pseudomonas syringae pathogens. PLoS One 8, e82445. https://doi.org/10.1371/journal.pone.0082445

Lee, S., Whitaker, V.M., Hutton, S.F., 2016. Mini Review: Potential Applications of Non-host Resistance for Crop Improvement. Front. Plant Sci. https://doi.org/10.3389/fpls.2016.00997

Li, C.F., Henderson, I.R., Song, L., Fedoroff, N., Lagrange, T., Jacobsen, S.E., 2008. Dynamic regulation of ARGONAUTE4 within multiple nuclear bodies in Arabidopsis thaliana. PLoS Genet. 4. https://doi.org/10.1371/journal.pgen.0040027

Li, C.F., Pontes, O., El-Shami, M., Henderson, I.R., Bernatavichute, Y. V., Chan, S.W.L., Lagrange, T., Pikaard, C.S., Jacobsen, S.E., 2006. An ARGONAUTE4-Containing Nuclear

Processing Center Colocalized with Cajal Bodies in Arabidopsis thaliana. Cell 126, 93–106. https://doi.org/10.1016/j.cell.2006.05.032

Li, F., Wang, A., 2019. RNA-Targeted Antiviral Immunity: More Than Just RNA Silencing. Trends Microbiol. xx, 1–14. https://doi.org/10.1016/j.tim.2019.05.007

Li, F., Zhao, N., Li, Z., Xu, X., Wang, Y., Yang, X., Liu, S.-S., Wang, A., Zhou, X., 2017. A calmodulin-like protein suppresses RNA silencing and promotes geminivirus infection by degrading SGS3 via the autophagy pathway in Nicotiana benthamiana. PLoS Pathog. 13, e1006213. https://doi.org/10.1371/journal.ppat.1006213

Liang Wu, Huan Chen, C.C.& Z.Q.F., 2014. Go in for the kill: How plants deploy effectortriggered immunity to combat pathogens. Virulence 5, 710–721. https://doi.org/10.4161/viru.29755

Liao, Y.-C., Pingli, H., Senzhao, C., Yao, M.-J., Zhang, J.-B., Liu, J.-L., Lia0, Y.-C., 2006. Biotechnology and Genetic Engineering Reviews Plantibodies: A Novel Strategy to Create Pathogen-Resistant Plants 13 Plantibodies: A Novel Strategy to Create Pathogen-Resistant Plants. Biotechnol Genet Eng Rev. 23, 253–71. https://doi.org/10.1080/02648725.2006.10648087

Lico, C., Benvenuto, E., Baschieri, S., 2015. The Two-Faced Potato Virus X: From Plant Pathogen to Smart Nanoparticle. Front. Plant Sci. 6, 1009. https://doi.org/10.3389/fpls.2015.01009

Liu, J., Carmell, M. a, Rivas, F. V, Marsden, C.G., Thomson, J.M., Song, J.-J., Hammond, S.M., Joshua-Tor, L., Hannon, G.J., 2004. Argonaute2 is the catalytic engine of mammalian RNAi. Science 305, 1437–1441. https://doi.org/10.1126/science.1102513

Liu, Q., Feng, Y., Zhu, Z., 2009. Dicer-like (DCL) proteins in plants. Funct. Integr. Genomics.

Lough, T.J., Lee, R.H., Emerson, S.J., Forster, R.L.S., Lucas, W.J., 2006. Functional analysis of the 5′ untranslated region of potexvirus RNA reveals a role in viral replication and cell-to-cell movement. Virology 351, 455–465. https://doi.org/10.1016/J.VIROL.2006.03.043

Lu, R., Malcuit, T., Moffett, P., Ruiz, M.T., Peart, J., Wu, A.J., Rathjen, J.P., Bendahmane, A., Day, L., Baulcombe, D.C., 2003. High throughput virus-induced gene silencing implicates

heat shock protein 90 in plant disease resistance. Embo J 22. https://doi.org/10.1093/emboj/cdg546

Lucht, J.M., 2015. Public Acceptance of Plant Biotechnology and GM Crops. Viruses 7, 4254–81. https://doi.org/10.3390/v7082819

Ludman, M., Burgyán, J., Fátyol, K., 2017a. Crispr/Cas9 Mediated Inactivation of Argonaute 2 Reveals its Differential Involvement in Antiviral Responses. Sci. Rep. 7, 1010. https://doi.org/10.1038/s41598-017-01050-6

Ludman, M., Burgyán, J., Fátyol, K., 2017b. Crispr/Cas9 Mediated Inactivation of Argonaute 2 Reveals its Differential Involvement in Antiviral Responses /631/337/384/2053 /631/449/2169/597 /42/89 /42/44 /38/22 article. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-01050-6

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P., Barton, M.K., 1999. The PINHEAD / ZWILLE gene acts pleiotropically in Arabidopsis development and has overlapping functions with the ARGONAUTE1 gene. Development 481, 469–481.

Ma, J.-B., Ye, K., Patel, D.J., 2004. Structural basis for overhang-specific small interfering RNA recognition by the PAZ domain. Nature 429, 318–22. https://doi.org/10.1038/nature02519

Ma, X., Nicole, M.-C., Meteignier, L.-V., Hong, N., Wang, G., Moffett, P., 2015. Different roles for RNA silencing and RNA processing components in virus recovery and virus-induced gene silencing in plants. J. Exp. Bot. 66, 919–932. https://doi.org/10.1093/jxb/eru447

Mallory, A., Vaucheret, H., 2010. Form, function, and regulation of ARGONAUTE proteins. Plant Cell 22, 3879–89. https://doi.org/10.1105/tpc.110.080671

Mallory, A.C., Hinze, A., Tucker, M.R., Bouche, N., Gasciolli, V., Elmayan, T., Lauressergues, D., Jauvion, V., Vaucheret, H., Laux, T., 2009. Redundant and Specific Roles of the ARGONAUTE Proteins AGO1 and ZLL in Development and Small RNA- Directed Gene Silencing. PLoS Genet. 5, 1–12. https://doi.org/10.1371/journal.pgen.1000646

Manavella, P.A., Koenig, D., Weigel, D., 2012. Plant secondary siRNA production determined by microRNA-duplex structure. Proc. Natl. Acad. Sci. U. S. A. 109, 2461–6.

Manzano-Piedras, E., Marcer, A., Alonso-Blanco, C., Picó, F.X., 2014. Deciphering the Adjustment between Environment and Life History in Annuals: Lessons from a Geographically-Explicit Approach in Arabidopsis thaliana. PLoS One 9, e87836. https://doi.org/10.1371/journal.pone.0087836

Matić, S., Noris, E., Contin, R., Marian, D., Thompson, J.R., 2019. Engineering partial resistance to cucumber mosaic virus in tobacco using intrabodies specific for the viral polymerase. Phytochemistry 162, 99–108. https://doi.org/10.1016/J.PHYTOCHEM.2019.03.006

Matzke, M. a, Kanno, T., Matzke, A.J.M., 2014. RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants. Annu. Rev. Plant Biol. 1–25. https://doi.org/10.1146/annurev-arplant-043014-114633

Matzke, M.A., Kanno, T., Matzke, A.J.M., 2015. RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants. Annu. Rev. Plant Biol. 66, 243–267. https://doi.org/10.1146/annurev-arplant-043014-114633

Maunoury, N., Vaucheret, H., 2011. AGO1 and AGO2 Act Redundantly in miR408-Mediated Plantacyanin Regulation. PLoS One 6, e28729. https://doi.org/10.1371/journal.pone.0028729

Mauricio, R., 1998. Costs of Resistance to Natural Enemies in Field Populations of the Annual Plant Arabidopsis thaliana Costs of Resistance to Natural Enemies in Field Populations of the Annual Plant Arabidopsis thaliana count for the common observation that, although plant. Source Am. Nat. 151, 20–28. https://doi.org/10.1086/286099

McCue, A.D., Panda, K., Nuthikattu, S., Choudury, S.G., Thomas, E.N., Slotkin, R.K., 2015. ARGONAUTE 6 bridges transposable element mRNA-derived siRNAs to the establishment of DNA methylation. EMBO J. 34, 20–35. https://doi.org/10.15252/embj.201489499

McHale, L., Tan, X., Koehl, P., Michelmore, R.W., 2006. Plant NBS-LRR proteins: adaptable guards. Genome Biol. 7, 212. https://doi.org/10.1186/gb-2006-7-4-212

Meister, G., 2013. Argonaute proteins: Functional insights and emerging roles. Nat. Rev. Genet. https://doi.org/10.1038/nrg3462

Méndez-Vigo, B., Gomaa, N.H., Alonso-Blanco, C., Xavier Picó, F., 2013. Among- and within-

population variation in flowering time of Iberian *Arabidopsis thaliana* estimated in field and glasshouse conditions. New Phytol. 197, 1332–1343. https://doi.org/10.1111/nph.12082

Méndez-Vigo, B., Picó, F.X., Ramiro, M., Martínez-Zapater, J.M., Alonso-Blanco, C., 2011. Altitudinal and Climatic Adaptation Is Mediated by Flowering Traits and FRI, FLC, and PHYC Genes in Arabidopsis. Plant Physiol. 157, 1942–1955. https://doi.org/10.1104/PP.111.183426

Mérai, Z., Kerényi, Z., Molnár, A., Barta, E., Válóczi, A., Bisztray, G., Havelda, Z., Burgyán, J., Silhavy, D., 2005. Aureusvirus P14 is an efficient RNA silencing suppressor that binds double-stranded RNAs without size specificity. J. Virol. 79, 7217–26. https://doi.org/10.1128/JVI.79.11.7217-7226.2005

Mi, S., Cai, T., Hu, Y., Chen, Y., Hodges, E., Ni, F., Wu, L., Li, S., Zhou, H., Long, C., Chen, S., Hannon, G.J., Qi, Y., 2008. Sorting of Small RNAs into Arabidopsis Argonaute Complexes Is Directed by the 5′ Terminal Nucleotide. Cell 133, 116–127. https://doi.org/10.1016/j.cell.2008.02.034

Mingot, A., Valli, A., Rodamilans, B., San León, D., Baulcombe, D.C., García, J.A., López-Moya, J.J., 2016. The P1N-PISPO trans -Frame Gene of Sweet Potato Feathery Mottle Potyvirus Is Produced during Virus Infection and Functions as an RNA Silencing Suppressor . J. Virol. 90, 3543–3557. https://doi.org/10.1128/jvi.02360-15

Miyashita, Y., Atsumi, G., Nakahara, K.S., 2016. Trade-Offs for Viruses in Overcoming Innate Immunities in Plants. Mol. Plant-Microbe Interact. 29, 595–598. https://doi.org/10.1094/MPMI-05-16-0103-CR

Mlotshwa, S., Pruss, G.J., Vance, V., 2008. Small RNAs in viral infection and host defense. Trends Plant Sci. 13, 375–382. https://doi.org/10.1016/j.tplants.2008.04.009

Molnar, A., Melnyk, C.W., Bassett, A., Hardcastle, T.J., Dunn, R., Baulcombe, D.C., 2010. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science 328, 872–875. https://doi.org/10.1126/science.1187959

Montavon, T., Kwon, Y., Zimmermann, A., Hammann, P., Vincent, T., Cognat, V., Michel, F., Dunoyer, P., 2017. A specific dsRNA-binding protein complex selectively sequesters

endogenous inverted-repeat siRNA precursors and inhibits their processing. Nucleic Acids Res. 45, 1330–1344. https://doi.org/10.1093/nar/gkw1264

Montes, N., Alonso-Blanco, C., García-Arenal, F., 2019. Cucumber mosaic virus infection as a potential selective pressure on Arabidopsis thaliana populations. PLOS Pathog. 15, e1007810. https://doi.org/10.1371/journal.ppat.1007810

Montgomery, T.A., Howell, M.D., Cuperus, J.T., Li, D., Hansen, J.E., Alexander, A.L., Chapman, E.J., Fahlgren, N., Allen, E., Carrington, J.C., 2008. Specificity of ARGONAUTE7-miR390 Interaction and Dual Functionality in TAS3 Trans-Acting siRNA Formation. Cell 133, 128–141. https://doi.org/10.1016/j.cell.2008.02.033

Morel, J., Godon, C., Mourrain, P., Béclin, C., Boutet, S., Feuerbach, F., Proux, F., Vaucheret, H., 2002. Fertile Hypomorphic *ARGONAUTE* (*ago1*) mutants impaired in post-transcriptional gene silencing and virus resistance. Society 14, 629–639. https://doi.org/10.1105/tpc.010358.are

Moreno, A.B., Martínez De Alba, A.E., Bardou, F., Crespi, M.D., Vaucheret, H., Maizel, A., Mallory, A.C., 2013. Cytoplasmic and nuclear quality control and turnover of single-stranded RNA modulate post-transcriptional gene silencing in plants. Nucleic Acids Res. 41, 4699–4708. https://doi.org/10.1093/nar/gkt152

Mourrain, P., Bé, C., Elmayan, T., Feuerbach, F., Godon, C., Morel, J.-B., Jouette, D., Lacombe, A.-M., Nikic, S., Picault, N., Ré, K., Sanial, M., Vo, T.-A., Vaucheret, H., 2000. Arabidopsis SGS2 and SGS3 Genes Are Required for Posttranscriptional Gene Silencing and Natural Virus Resistance gene required for PTGS, a possible mechanistic link. Cell 101, 533–542.

Moussian, B., Schoof, H., Haecker, A., Jurgens, G., Laux, T., 1998. Role of the ZWILLE gene in the regulation of central shoot meristem cell fate during Arabidopsis embryogenesis. EMBO J. 17, 1799–1809.

Muangsan, N., Beclin, C., Vaucheret, H., Robertson, D., 2004. Geminivirus VIGS of endogenous genes requires SGS2/SDE1 and SGS3 and defines a new branch in the genetic pathway for silencing in plants. Plant J. 38, 1004–1014. https://doi.org/10.1111/j.1365-

313X.2004.02103.x

Mukherjee, K., Campos, H., Kolaczkowski, B., 2013. Evolution of animal and plant dicers: Early parallel duplications and recurrent adaptation of antiviral RNA binding in plants. Mol. Biol. Evol. 30, 627–641. https://doi.org/10.1093/molbev/mss263

Musiyenko, A., Majumdar, T., Andrews, J., Adams, B., Barik, S., 2012. PRMT1 methylates the single Argonaute of Toxoplasma gondii and is important for the recruitment of Tudor nuclease for target RNA cleavage by antisense guide RNA. Cell. Microbiol. 14, 882–901. https://doi.org/10.1111/j.1462-5822.2012.01763.x

Nakasugi, K., Crowhurst, R.N., Bally, J., Wood, C.C., Hellens, R.P., Waterhouse, P.M., 2013. De novo transcriptome sequence assembly and analysis of RNA silencing genes of Nicotiana benthamiana. PLoS One 8, e59534. https://doi.org/10.1371/journal.pone.0059534

Naylor, M., Murphy, A.M., Berry, J.O., Carr, J.P., 1998. Salicylic Acid Can Induce Resistance to Plant Virus Movement 11, 860–868.

Nguyen, H.D., Tomitaka, Y., Ho, S.Y.W., Duchêne, S., Vetten, H.-J., Lesemann, D., Walsh, J.A., Gibbs, A.J., Ohshima, K., 2013. Turnip Mosaic Potyvirus Probably First Spread to Eurasian Brassica Crops from Wild Orchids about 1000 Years Ago. PLoS One 8, e55336. https://doi.org/10.1371/journal.pone.0055336

Noah Fahlgren, T.A.M., Miya D. Howell,Edwards Allen, Sarah K. Dvorak, Amanda L. Alexander, and J.C.C., 2006. Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA Affects Developmental Timing and Patterning in Arabidopsis. Curr. Biol. 16, 939–944. https://doi.org/10.1016/j.cub.2006.03.065

Nonomura, K.-I., Morohoshi, A., Nakano, M., Eiguchi, M., Miyao, A., Hirochika, H., Kurata, N., 2007. A Germ Cell–Specific Gene of the ARGONAUTE Family Is Essential for the Progression of Premeiotic Mitosis and Meiosis during Sporogenesis in Rice. Plant Cell 19, 2583–2594. https://doi.org/10.1105/tpc.107.053199

Nordborg, M., Hu, T.T., Ishino, Y., Jhaveri, J., Toomajian, C., Zheng, H., Bakker, E., Calabrese, P., Gladstone, J., Goyal, R., Jakobsson, M., Kim, S., Morozov, Y., Padhukasahasram, B., Plagnol, V., Rosenberg, N.A., Shah, C., Wall, J.D., Wang, J., Zhao, K., Kalbfleisch, T.,

Schulz, V., Kreitman, M., Bergelson, J., 2005. The pattern of polymorphism in Arabidopsis thaliana. PLoS Biol. 3, 1289–1299. https://doi.org/10.1371/journal.pbio.0030196

Nuthikattu, S., McCue, A.D., Panda, K., Fultz, D., DeFraia, C., Thomas, E.N., Slotkin, R.K., 2013. The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21-22 nucleotide small interfering RNAs. Plant Physiol. 162, 116–31. https://doi.org/10.1104/pp.113.216481

Oa, W., Garcia-Ruiz, H., Takeda, A., Chapman, E.J., Sullivan, C.M., Fahlgren, N., Brempelis, K.J., Carrington, J.C., 2010. Arabidopsis RNA-Dependent RNA Polymerases and Dicer-Like Proteins in Antiviral Defense and Small Interfering RNA Biogenesis during Turnip Mosaic Virus Infection. Plant Cell 22, 481–496. https://doi.org/10.1105/tpc.109.073056

Obbard, D.J., Jiggins, F.M., Halligan, D.L., Little, T.J., 2006. Natural selection drives extremely rapid evolution in antiviral RNAi genes. Curr. Biol. 16, 580–585. https://doi.org/10.1016/j.cub.2006.01.065

Odokonyero, D., Mendoza, M.R., Alvarado, V.Y., Zhang, J., Wang, X., Scholthof, H.B., 2015. Transgenic down-regulation of ARGONAUTE2 expression in Nicotiana benthamiana interferes with several layers of antiviral defenses. Virology 486, 209–218. https://doi.org/10.1016/j.virol.2015.09.008

Odokonyero, D., Mendoza, M.R., Moffett, P., Scholthof, H.B., 2017. *Tobacco rattle virus* (TRV)-Mediated Silencing of *Nicotiana benthamiana ARGONAUTES* (*NbAGOs*) Reveals New Antiviral Candidates and Dominant Effects of TRV- *NbAGO1*. Phytopathology PHYTO-02-17-004. https://doi.org/10.1094/PHYTO-02-17-0049-R

Okano, Y., Senshu, H., Hashimoto, M., Neriya, Y., Netsu, O., Minato, N., Yoshida, T., Maejima, K., Oshima, K., Komatsu, K., Yamaji, Y., Namba, S., 2014. In Planta Recognition of a Double-Stranded RNA Synthesis Protein Complex by a Potexviral RNA Silencing Suppressor. Plant Cell 26, 2168–2183. https://doi.org/10.1105/tpc.113.120535

Olmedo-Monfil, V., Durán-Figueroa, N., Arteaga-Vázquez, M., Demesa-Arévalo, E., Autran, D., Grimanelli, D., Slotkin, R.K., Martienssen, R.A., Vielle-Calzada, J.-P., 2010. Control of female gamete formation by a small RNA pathway in Arabidopsis. Nature 464, 628–632.

https://doi.org/10.1038/nature08828

Olspert, A., Chung, B.Y., Atkins, J.F., Carr, J.P., Firth, A.E., 2015. Transcriptional slippage in the positive-sense RNA virus family Potyviridae. EMBO Rep. 16, 995–1004. https://doi.org/10.15252/embr.201540509

Omarov, R.T., Ciomperlik, J., Scholthof, H.B., 2016. An in vitro reprogrammable antiviral RISC with size-preferential ribonuclease activity. Virology 490, 41–48. https://doi.org/10.1016/J.VIROL.2015.12.017

Pagán, I., Alonso-Blanco, C., García-Arenal, F., 2009. Differential Tolerance to Direct and Indirect Density-Dependent Costs of Viral Infection in Arabidopsis thaliana. PLoS Pathog. 5, e1000531. https://doi.org/10.1371/journal.ppat.1000531

Pagán, I., Alonso-Blanco, C., García-Arenal, F., 2008. Host Responses in Life-History Traits and Tolerance to Virus Infection in Arabidopsis thaliana. PLoS Pathog. 4, e1000124. https://doi.org/10.1371/journal.ppat.1000124

Pagán, I., Fraile, A., Fernandez-Fueyo, E., Montes, N., Alonso-Blanco, C., García-Arenal, F., 2010a. Arabidopsis thaliana as a model for the study of plant-virus co-evolution. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 365, 1983–95. https://doi.org/10.1098/rstb.2010.0062

Pagán, I., Fraile, A., Fernandez-Fueyo, E., Montes, N., Alonso-Blanco, C., García-Arenal, F., 2010b. Arabidopsis thaliana as a model for the study of plant–virus co-evolution. Philos. Trans. R. Soc. B Biol. Sci. 365, 1983–1995. https://doi.org/10.1098/rstb.2010.0062

Pagán, I., García-Arenal, F., 2018. Tolerance to Plant Pathogens: Theory and Experimental Evidence. Int. J. Mol. Sci. 19, 810. https://doi.org/10.3390/ijms19030810

Palukaitis, P., Roossinck, M.J., Dietzgen, R.G., Francki, R.I.B., 1992. Cucumber MOSAIC Virus. Adv. Virus Res. 41, 281–348. https://doi.org/10.1016/S0065-3527(08)60039-1

Parent, J.-S., Bouteiller, N., Elmayan, T., Vaucheret, H., 2015. Respective contributions of Arabidopsis DCL2 and DCL4 to RNA silencing. Plant J. 81, 223–232. https://doi.org/10.1111/tpj.12720

Paudel, D.B., Ghoshal, B., Jossey, S., Ludman, M., Fatyol, K., Sanfaçon, H., 2018. Expression

and antiviral function of ARGONAUTE 2 in Nicotiana benthamiana plants infected with two isolates of tomato ringspot virus with varying degrees of virulence. Virology 524, 127–139. https://doi.org/10.1016/J.VIROL.2018.08.016

Paudel, D.B., Sanfaçon, H., 2018. Exploring the Diversity of Mechanisms Associated With Plant Tolerance to Virus Infection. Front. Plant Sci. 9, 1575. https://doi.org/10.3389/fpls.2018.01575

Paulo, J., Machado, B., Calil, I.P., Santos, A.A., Fontes, E.P.B., 2017. Translational control in plant antiviral immunity. Genet. Mol. Biol. 40, 292–304. https://doi.org/10.1590/1678-4685-GMB-2016-0092

Peart, J.R., Cook, G., Feys, B.J., Parker, J.E., Baulcombe, D.C., 2002a. An EDS1 orthologue is required for N-mediated resistance against tobacco mosaic virus. Plant J. 29, 569–579. https://doi.org/10.1046/j.1365-313X.2002.029005569.x

Peart, J.R., Lu, R., Sadanandom, A., Malcuit, I., Moffett, P., Brice, D.C., Schauser, L., Jaggard, D.A.W., Xiao, S., Coleman, M.J., Dow, M., Jones, J.D.G., Shirasu, K., Baulcombe, D.C., 2002b. Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. Proc. Natl. Acad. Sci. U. S. A. 99, 10865–10869. https://doi.org/10.1073/pnas.152330599

Picó, F.X., Méndez-Vigo, B., Martínez-Zapater, J.M., Alonso-Blanco, C., 2008. Natural Genetic Variation of Arabidopsis thaliana Is Geographically Structured in the Iberian Peninsula. Genetics 180, 1009–1021. https://doi.org/10.1534/genetics.108.089581

Pieterse, C.M.J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., Van Wees, S.C.M., 2012. Hormonal Modulation of Plant Immunity. Annu. Rev. Cell Dev. Biol. 28, 489–521. https://doi.org/10.1146/annurev-cellbio-092910-154055

Pita, J.S., Morris, V., Roossinck, M.J., 2015. Mutation and Recombination Frequencies Reveal a Biological Contrast within Strains of Cucumber Mosaic Virus Downloaded from. J Virol 89, 6817–6823. https://doi.org/10.1128/JVI.01891-12

Pogany, J., Nagy, P.D., 2015. Activation of *Tomato Bushy Stunt Virus* RNA-Dependent RNA Polymerase by Cellular Heat Shock Protein 70 Is Enhanced by Phospholipids *In Vitro*. J.

Virol. 89, 5714–5723. https://doi.org/10.1128/JVI.03711-14

Pogany, J., Nagy, P.D., 2012. p33-Independent Activation of a Truncated p92 RNA-Dependent RNA Polymerase of Tomato Bushy Stunt Virus in Yeast Cell-Free Extract. J. Virol. 86, 12025–12038.

Pontier, D., Picart, C., Roudier, F.O., Garcia, D., Lahmy, S., Azevedo, J., Alart, E., Le Laudié, M., Karlowski, W.M., Cooke, R., Colot, V., Voinnet, O., Lagrange, T., 2012. Molecular Cell Article NERD, a Plant-Specific GW Protein, Defines an Additional RNAi-Dependent Chromatin-Based Pathway in Arabidopsis. https://doi.org/10.1016/j.molcel.2012.07.027

Poulsen, C., Vaucheret, H., Brodersen, P., 2013. Lessons on RNA silencing mechanisms in plants from eukaryotic argonaute structures. Plant Cell 25, 22–37. https://doi.org/10.1105/tpc.112.105643

Powers, J.G., Sit, T.L., Qu, F., Morris, T.J., Kim, K.-H., Lommel, S.A., 2008. A Versatile Assay for the Identification of RNA Silencing Suppressors Based on Complementation of Viral Movement 21, 879–890.

Pradhan, M., Pandey, P., Gase, K., Sharaff, M., Singh, R., Sethi, A., Baldwin, I.T., Pandey, S.P., 2017. AGO8 mediates defense response against herbivores 2. Plant Physiol. https://doi.org/10.1104/pp.17.00702

Pumplin, N., Voinnet, O., 2013. RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence. Nat. Rev. Microbiol. 11, 745–60.

Qi, X., Bao, F.S., Xie, Z., 2009. Small RNA deep sequencing reveals role for Arabidopsis thaliana RNA-dependent RNA polymerases in viral siRNA biogenesis. PLoS One 4, e4971. https://doi.org/10.1371/journal.pone.0004971

Qi, Y., Denli, A.M., Hannon, G.J., 2005. Technique Biochemical Specialization within Arabidopsis RNA Silencing Pathways. Mol. Cell 19, 421–428. https://doi.org/10.1016/j.molcel.2005.06.014

Qu, F., Morris, T.J., 2005. Suppressors of RNA silencing encoded by plant viruses and their role in viral infections. FEBS Lett.

Qu, F., Ye, X., Morris, T.J., 2008. Arabidopsis DRB4, AGO1, AGO7, and RDR6 participate in a DCL4-initiated antiviral RNA silencing pathway negatively regulated by DCL1. Proc. Natl. Acad. Sci. U. S. A. 105, 14732–7. https://doi.org/10.1073/pnas.0805760105

Raja, Priya, Jackel, J.N., Li, S., Heard, I.M., Bisaro, D.M., 2014. Arabidopsis double-stranded RNA binding protein DRB3 participates in methylation-mediated defense against geminiviruses. J. Virol. 88, 2611–22. https://doi.org/10.1128/JVI.02305-13

Raja, P., Jackel, J.N., Li, S., Heard, I.M., Bisaro, D.M., 2014. Arabidopsis Double-Stranded RNA Binding Protein DRB3 Participates in Methylation-Mediated Defense against Geminiviruses. J. Virol. 88, 2611–2622. https://doi.org/10.1128/JVI.02305-13

Raja, P., Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008a. Viral Genome Methylation as an Epigenetic Defense against Geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, Priya, Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008. Viral genome methylation as an epigenetic defense against geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, P., Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008b. Viral Genome Methylation as an Epigenetic Defense against Geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, P., Wolf, J.N., Bisaro, D.M., 2010. RNA silencing directed against geminiviruses: Post-transcriptional and epigenetic components. Biochim. Biophys. Acta - Gene Regul. Mech. 1799, 337–351. https://doi.org/10.1016/j.bbagrm.2010.01.004

Rambod Abiri, al, Valdiani, A., Maziah, M., Azmi Shaharuddin, N., Sahebi, M., Norhana Balia Yusof, Z., Atabaki, N., Talei, D., Valdiani alireza, A., 2016. A Critical Review of the Concept of Transgenic Plants: Insights into Pharmaceutical Biotechnology and Molecular Farming. Curr. Issues Mol. Biol. 18, 21–42. https://doi.org/10.21775/cimb.018.021

Reinhart, B.J., Weinstein, E.G., Rhoades, M.W., Bartel, B., Bartel, D.P., 2002. MicroRNAs in plants. Genes Dev.

Revers, F., García, J.A., 2015a. Molecular biology of potyviruses, in: Advances in Virus

Research. Academic Press Inc., pp. 101–199. https://doi.org/10.1016/bs.aivir.2014.11.006

Revers, F., García, J.A., 2015b. Molecular Biology of Potyviruses. Adv. Virus Res. 92, 101–199. https://doi.org/10.1016/BS.AIVIR.2014.11.006

Reyero-Saavedra, M. del R., Qiao, Z., Sánchez-Correa, M. del S., Reyes, J.L., Covarrubias, A.A., Libault, M., Valdés-López, O., 2017. Gene Silencing of Argonaute5 Negatively Affects the Establishment of the Legume-Rhizobia Symbiosis. Genes (Basel). 5, 1–19. https://doi.org/10.3390/genes8120352

Rivas, F. V, Tolia, N.H., Song, J.-J., Aragon, J.P., Liu, J., Hannon, G.J., Joshua-Tor, L., 2005. Purified Argonaute2 and an siRNA form recombinant human RISC. Nat. Struct. Mol. Biol. 12, 340–349. https://doi.org/10.1038/nsmb918

Rodríguez-Leal, D., Castillo-Cobián, A., Rodríguez-Arévalo, I., Vielle-Calzada, J.-P., 2016. A Primary Sequence Analysis of the ARGONAUTE Protein Family in Plants. Front. Plant Sci. 7, 1347. https://doi.org/10.3389/fpls.2016.01347

Roossinck, M.J., 2002. Evolutionary history of Cucumber mosaic virus deduced by phylogenetic analyses. J. Virol. 76, 3382–7. https://doi.org/10.1128/jvi.76.7.3382-3387.2002

Roossinck, M.J., 2001. Cucumber mosaic virus, a model for RNA virus evolution. Mol. Plant Pathol. 2, 59–63. https://doi.org/10.1046/j.1364-3703.2001.00058.x

Rosa, C., Kuo, Y.-W., Wuriyanghan, H., Falk, B.W., 2018. RNA Interference Mechanisms and Applications in Plant Pathology. Annu. Rev. Phytopathol. 56, 581–610. https://doi.org/10.1146/annurev-phyto-080417

Rose, L., Atwell, S., Grant, M., Holub, E.B., 2012. Parallel Loss-of-Function at the RPM1 Bacterial Resistance Locus in Arabidopsis thaliana. Front. Plant Sci. 3, 287. https://doi.org/10.3389/fpls.2012.00287

Roth, B.M., Pruss, G.J., Vance, V.B., 2004. Review-Plant viral suppressors of RNA silencing. Virus Res. 102, 97–108. https://doi.org/10.1016/j.virusres.2004.01.020

Sanjuán, R., Nebot, M.R., Chirico, N., Louis, M., Belshaw, R., Sanjua, R., Mansky, L.M., 2010. Viral Mutation Rates Viral Mutation Rates □. J. Virol. 84, 9733–9748.

https://doi.org/10.1128/JVI.00694-10

Saumet, A., Lecellier, C.-H., 2006. Anti-viral RNA silencing: do we look like plants? Retrovirology 3, 3. https://doi.org/10.1186/1742-4690-3-3

Schepetilnikov, M. V., Manske, U., Solovyev, A.G., Jr, A.A.Z., Schiemann, J., Morozov, S.Y., 2005. The hydrophobic segment of Potato virus X TGBp3 is a major determinant of the protein intracellular trafficking. J. Gen. Virol. 86, 2379–2391. https://doi.org/10.1099/vir.0.80865-0

Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Schölkopf, B., Weigel, D., Lohmann, J.U., 2005. A gene expression map of Arabidopsis thaliana development. Nat. Genet. 37, 501–506. https://doi.org/10.1038/ng1543

Scholthof, H.B., Alvarado, V.Y., Vega-Arreguin, J.C., Ciomperlik, J., Odokonyero, D., Brosseau, C., Jaubert, M., Zamora, A., Moffett, P., 2011. Identification of an ARGONAUTE for antiviral RNA silencing in Nicotiana benthamiana. Plant Physiol. 156, 1548–55. https://doi.org/10.1104/pp.111.178764

Schott, G., Mari-Ordonez, A., Himber, C., Alioua, A., Voinnet, O., Dunoyer, P., 2012. Differential effects of viral silencing suppressors on siRNA and miRNA loading support the existence of two distinct cellular pools of ARGONAUTE1. EMBO J. 31, 2553–65. https://doi.org/10.1038/emboj.2012.92

Schuck, J., Gursinsky, T., Pantaleo, V., Burgy??n, J., Behrens, S.E., 2013a. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic Acids Res. 41, 5090–5103.

Schuck, J., Gursinsky, T., Pantaleo, V., Burgyán, J., Behrens, S.-E., 2013b. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic Acids Res. 41, 5090–103. https://doi.org/10.1093/nar/gkt193

Senshu, H., Ozeki, J., Komatsu, K., Hashimoto, M., Hatada, K., Aoyama, M., Kagiwada, S., Yamaji, Y., Namba, S., 2017. Variability in the level of RNA silencing suppression caused by triple gene block protein 1 (TGBp1) from various potexviruses during infection. https://doi.org/10.1099/vir.0.008243-0

Senshu, H., Ozeki, J., Komatsu, K., Hashimoto, M., Hatada, K., Aoyama, M., Kagiwada, S., Yamaji, Y., Namba, S., 2009. Variability in the level of RNA silencing suppression caused by triple gene block protein 1 (TGBp1) from various potexviruses during infection. J. Gen. Virol. https://doi.org/10.1099/vir.0.008243-0

Senthil-Kumar, M., Mysore, K.S., 2013. Nonhost Resistance Against Bacterial Pathogens: Retrospectives and Prospects. Annu. Rev. Phytopathol. https://doi.org/10.1146/annurev-phyto-082712-102319

Shamandi, N., Zytnicki, M., Charbonnel, C., Elvira-Matelot, E., Bochnakian, A., Comella, P., Mallory, A.C., Lepère, G., Sáez-Vásquez, J., Vaucheret, H., 2015a. Plants Encode a General siRNA Suppressor That Is Induced and Suppressed by Viruses. PLoS Biol. 13.

Shamandi, N., Zytnicki, M., Charbonnel, C., Elvira-Matelot, E., Bochnakian, A., Comella, P., Mallory, A.C., Lepère, G., Sáez-Vásquez, J., Vaucheret, H., 2015b. Plants Encode a General siRNA Suppressor That Is Induced and Suppressed by Viruses. PLOS Biol. 13, e1002326. https://doi.org/10.1371/journal.pbio.1002326

Shen, Q., Hu, T., Bao, M., Cao, L., Zhang, H., Song, F., Xie, Q., Zhou, X., 2016. Tobacco RING E3 Ligase NtRFP1 Mediates Ubiquitination and Proteasomal Degradation of a Geminivirus-Encoded βC1. Mol. Plant 9, 911–925. https://doi.org/10.1016/j.molp.2016.03.008

Shukla, A., Pagán, I., García-Arenal, F., 2018. Effective tolerance based on resource reallocation is a virus-specific defence in *Arabidopsis thaliana*. Mol. Plant Pathol. 19, 1454–1465. https://doi.org/10.1111/mpp.12629

Silhavy, D., Molnár, A., Lucioli, A., Szittya, G., Hornyik, C., Tavazza, M., Burgyán, J., 2002. A viral protein suppresses RNA silencing and binds silencing-generated, 21- to 25-nucleotide double-stranded RNAs. EMBO J. 21, 3070–80. https://doi.org/10.1093/emboj/cdf312

Siomi, M.C., Mannen, T., Siomi, H., 2010. How does the royal family of tudor rule the PIWI-interacting RNA pathway? Genes Dev. 24, 636–646. https://doi.org/10.1101/gad.1899210

Solovyev, A.G., Kalinina, N.O., Morozov, S.Y., 2012. Recent Advances in Research of Plant Virus Movement Mediated by Triple Gene Block. Front. Plant Sci. https://doi.org/10.3389/fpls.2012.00276

Solovyev, A.G., Stroganova, T.A., Zamyatnin, A.A., Fedorkin, O.N., Schiemann, J., Morozov, S.Y., 2000. Subcellular Sorting of Small Membrane-Associated Triple Gene Block Proteins: TGBp3-Assisted Targeting of TGBp2. Virology 269, 113–127. https://doi.org/10.1006/VIRO.2000.0200

Song, J.-J., Smith, S.K., HannonL, G.J., Joshua-Tor, L., 2004. Crystal Structure of Argonaute and Its Implications for RISC Slicer Activity. Science (80-.). 305, 1434–1437. https://doi.org/10.1126/science.1102514

Song, M.-S., Rossi, J.J., 2017. Molecular mechanisms of Dicer: endonuclease and enzymatic activity. Biochem. J. 474, 1603–1618. https://doi.org/10.1042/bcj20160759

Stael, S., Kmiecik, P., Willems, P., Van Der Kelen, K., Coll, N.S., Teige, M., Van Breusegem, F., 2015. Plant innate immunity--sunny side up? Trends Plant Sci. 20, 3–11. https://doi.org/10.1016/j.tplants.2014.10.002

Stahl, E.A., Dwyer, G., Mauricio, R., Kreitman, M., Bergelson, J., 1999. Dynamics of disease resistance polymorphism at the Rpm1 locus of Arabidopsis. Nature 400, 667–671. https://doi.org/10.1038/23260

Sun, Y., Sparks, C., Jones, H., Riley, M., Francis, F., Du, W., Xia, L., 2019. Silencing an essential gene involved in infestation and digestion in grain aphid through plant-mediated interference generates aphid-resistant wheat plants. Plant Biotechnol. J. 17, 852–854. https://doi.org/10.1111/pbi.13067

Syller, J., 2012. Facilitative and antagonistic interactions between plant viruses in mixed infections. Mol. Plant Pathol. https://doi.org/10.1111/j.1364-3703.2011.00734.x

Szittya, G., Burgyán, J., 2013. RNA Interference-Mediated Intrinsic Antiviral Immunity in Plants. https://doi.org/10.1007/978-3-642-37765-5_6

Szittya, G., Moxon, S., Pantaleo, V., Toth, G., Rusholme Pilcher, R.L., Moulton, V., Burgyan, J., Dalmay, T., 2010. Structural and functional analysis of viral siRNAs. PLoS Pathog. 6, e1000838. https://doi.org/10.1371/journal.ppat.1000838

Takahashi, H., Miller, J., Nozaki, Y., Takeda, M., Shah, J., Hase, S., Ikegami, M., Ehara, Y., Dinesh-Kumar, S.P., 2002. RCY1, an Arabidopsis thaliana RPP8/HRT family resistance

gene, conferring resistance to cucumber mosaic virus requires salicylic acid, ethylene and a novel signal transduction mechanism. Plant J. 32, 655–667. https://doi.org/10.1046/j.1365-313X.2002.01453.x

Takahashi, H., Suzuki, M., Natsuaki, K., Shigyo, T., Hino, K., Teraoka, T., Hosokawa, D., Ehara, Y., 2001. Mapping the virus and host genes involved in the resistance response in cucumber mosaic virus-Infected Arabidopsis thaliana. Plant Cell Physiol. 42, 340–7.

Takeda, A., Iwasaki, S., Watanabe, T., Utsumi, M., Watanabe, Y., 2008. The Mechanism Selecting the Guide Strand from Small RNA Duplexes is Different Among Argonaute Proteins. Plant Cell Physiol. 49, 493–500. https://doi.org/10.1093/pcp/pcn043

Tecson Mendoza, E.M., C. Laurena, A., Botella, J.R., 2008. Recent advances in the development of transgenic papaya technology. Biotechnol. Annu. Rev. 14, 423–462. https://doi.org/10.1016/S1387-2656(08)00019-7

Thomas-Sharma, S., Abdurahman, A., Ali, S., Andrade-Piedra, J.L., Bao, S., Charkowski, A.O., Crook, D., Kadian, M., Kromann, P., Struik, P.C., Torrance, L., Garrett, K.A., Forbes, G.A., 2016. Seed degeneration in potato: the need for an integrated seed health strategy to mitigate the problem in developing countries. Plant Pathol. 65, 3–16. https://doi.org/10.1111/ppa.12439

Thompson, K., Stewart, A.J.A., 1981. The Measurement and Meaning of Reproductive Effort in Plants. Am. Nat. 117, 205–211. https://doi.org/10.1086/283700

Tilsner, J., Linnik, O., Wright, K.M., Bell, K., Roberts, A.G., Lacomme, C., Santa Cruz, S., Oparka, K.J., 2012. The TGB1 movement protein of Potato virus X reorganizes actin and endomembranes into the X-body, a viral replication factory. Plant Physiol. 158, 1359–70. https://doi.org/10.1104/pp.111.189605

Todesco, M., Balasubramanian, S., Hu, T.T., Traw, M.B., Horton, M., Epple, P., Kuhns, C., Sureshkumar, S., Schwartz, C., Lanz, C., Laitinen, R.A.E., Huang, Y., Chory, J., Lipka, V., Borevitz, J.O., Dangl, J.L., Bergelson, J., Nordborg, M., Weigel, D., 2010. Natural allelic variation underlying a major fitness trade-off in Arabidopsis thaliana. Nature 465. https://doi.org/10.1038/nature09083

Törjék, O., Meyer, R.C., Zehnsdorf, M., Teltow, M., Strompen, G., Witucka-Wall, H., Blacha, A., Altmann, T., 2008. Construction and Analysis of 2 Reciprocal Arabidopsis Introgression Line Populations. J. Hered. 99, 396–406. https://doi.org/10.1093/jhered/esn014

Tsuzuki, M., Takeda, A., Watanabe, Y., 2014. Recovery of dicer-like 1 -late flowering phenotype by miR172 expressed by the noncanonical DCL4-dependent biogenesis pathway. RNA 20, 1320–1327. https://doi.org/10.1261/rna.044966.114

Tucker, M.R., Okada, T., Hu, Y., Scholefield, a., Taylor, J.M., Koltunow, a. M.G., 2012. Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in Arabidopsis. Development 139, 1399–1404. https://doi.org/10.1242/dev.075390

Vagin, V. V, Wohlschlegel, J., Qu, J., Jonsson, Z., Huang, X., Chuma, S., Girard, A., Sachidanandam, R., Hannon, G.J., Aravin, A.A., 2009. methylation in specifying interaction with Tudor family members Proteomic analysis of murine Piwi proteins reveals a role for arginine methylation in specifying interaction with Tudor family members 1749–1762. https://doi.org/10.1101/gad.1814809

Vance, V., 2001. RNA Silencing in Plants--Defense and Counterdefense. Science (80-.). 292, 2277–2280. https://doi.org/10.1126/science.1061334

Várallyay, É., Oláh, E., Havelda, Z., 2014. Independent parallel functions of p19 plant viral suppressor of RNA silencing required for effective suppressor activity. Nucleic Acids Res. 42, 599–608. https://doi.org/10.1093/nar/gkt846

Varma, A. and M.N., 2001. Durable host-plant resistance, a desirable trait for integrated disease management.

Varsha Wesley, S., Helliwell, C.A., Smith, N.A., Wang, M., Rouse, D.T., Liu, Q., Gooding, P.S., Singh, S.P., Abbott, D., Stoutjesdijk, P.A., Robinson, S.P., Gleave, A.P., Green, A.G., Waterhouse, P.M., 2001. Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J. 27, 581–590. https://doi.org/10.1046/j.1365-313X.2001.01105.x

Vaucheret, H., 2008. Plant ARGONAUTES. Trends Plant Sci.

Vaucheret, H., Mallory, A.C., Bartel, D.P., 2006. AGO1 Homeostasis Entails Coexpression of

MIR168 and AGO1 and Preferential Stabilization of miR168 by AGO1. Mol. Cell 22, 129–136.

Vaucheret, H., Vazquez, F., Crété, P., Bartel, D.P., 2004. The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. Genes Dev. 18, 1187–1197. https://doi.org/10.1101/gad.1201404

Verchot-Lubicz, J., Torrance, L., Solovyev, A.G., Morozov, S.Y., Jackson, A.O., Gilmer, D., 2010. Varied Movement Strategies Employed by Triple Gene Block–Encoding Viruses 23, 1231–1247. https://doi.org/10.1094

Verlaan, M.G., Hutton, S.F., Ibrahem, R.M., Kormelink, R., Visser, R.G.F., Scott, J.W., Edwards, J.D., Bai, Y., 2013. The Tomato Yellow Leaf Curl Virus Resistance Genes Ty-1 and Ty-3 Are Allelic and Code for DFDGD-Class RNA-Dependent RNA Polymerases. PLoS Genet. 9.

Vickers, T.A., Koo, S., Bennett, C.F., Crooke, S.T., Dean, N.M., Baker, B.F., 2003. Efficient reduction of target RNAs by small interfering RNA and RNase H-dependent antisense agents. A comparative analysis. J. Biol. Chem. 278, 7108–7118.

Voinnet, O., Lederer, C., Baulcombe, D.C., 2000. A Viral Movement Protein Prevents Spread of the Gene Silencing Signal in Nicotiana benthamiana. Cell 103, 157–167. https://doi.org/10.1016/S0092-8674(00)00095-7

Voinnet, O., Pinto, Y.M., Baulcombe, D.C., Lohuis, D., Visser, R.G.F., Bai, Y., Kormelink, R., 1999. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. Proc. Natl. Acad. Sci. U. S. A. 96, 14147–52. https://doi.org/10.1073/pnas.96.24.14147

Wang, D., Liu, Q., Jones, H.D., Bruce, T., Xia, L., 2014. Comparative transcriptomic analyses revealed divergences of two agriculturally important aphid species. BMC Genomics 15. https://doi.org/10.1186/1471-2164-15-1023

Wang, J., Mei, J., Ren, G., 2019. Plant microRNAs: Biogenesis, Homeostasis, and Degradation. Front. Plant Sci. 10, 360. https://doi.org/10.3389/fpls.2019.00360

Wang, M.-B., Metzlaff, M., 2005. RNA silencing and antiviral defense in plants. Curr. Opin.

Plant Biol. 8, 216–222. https://doi.org/10.1016/j.pbi.2005.01.006

Wang, X.-B., Jovel, J., Udomporn, P., Wang, Y., Wu, Q., Li, W.-X., Gasciolli, V., Vaucheret, H., Ding, S.-W., 2011. The 21-Nucleotide, but Not 22-Nucleotide, Viral Secondary Small Interfering RNAs Direct Potent Antiviral Defense by Two Cooperative Argonautes in Arabidopsis thaliana. Plant Cell 23, 1625–1638. https://doi.org/10.1105/tpc.110.082305

Wang, X.-B., Wu, Q., Ito, T., Cillo, F., Li, W.-X., Chen, X., Yu, J.-L., Ding, S.-W., 2010. RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 107, 484–489. https://doi.org/10.1073/pnas.0904086107

Wassenegger, M., Krczal, G., 2006. Nomenclature and functions of RNA-directed RNA polymerases. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2006.01.003

Wei, T., Wang, L., Zhou, X., Ren, X., Dai, X., Liu, H., 2015. PopW activates PAMP-triggered immunity in controlling tomato bacterial spot disease. Biochem. Biophys. Res. Commun. 463, 746–750.

Wei, W., Ba, Z., Gao, M., Wu, Y., Ma, Y., Amiard, S., White, C.I., Rendtlew Danielsen, J.M., Yang, Y.-G., Qi, Y., 2012. A Role for Small RNAs in DNA Double-Strand Break Repair. Cell 149, 101–112. https://doi.org/10.1016/j.cell.2012.03.002

Willmann, M.R., Endres, M.W., Cook, R.T., Gregory, B.D., 2011. The Functions of RNA-Dependent RNA Polymerases in Arabidopsis. Arab. B. 9, e0146. https://doi.org/10.1199/tab.0146

Wu, J., Yang, Z., Wang, Y., Zheng, L., Ye, R., Ji, Y., Zhao, S., Ji, S., Liu, R., Xu, L., Zheng, H., Zhou, Y., Zhang, X., Cao, X., Xie, L., Wu, Z., Qi, Y., Li, Y., 2015. Viral-inducible Argonaute18 confers broad-spectrum virus resistance in rice by sequestering a host microRNA. Elife 4. https://doi.org/10.7554/eLife.05733

Xiao, Y., Wu, K., 2019. Recent progress on the interaction between insects and Bacillus thuringiensis crops. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2018.0316

Xie, Z., Johansen, L.K., Gustafson, A.M., Kasschau, K.D., Lellis, A.D., Zilberman, D., Jacobsen,

S.E., Carrington, J.C., 2004. Genetic and functional diversification of small RNA pathways in plants. PLoS Biol. 2, E104. https://doi.org/10.1371/journal.pbio.0020104

Xu, D., Zhou, G., 2017. Characteristics of siRNAs derived from Southern rice black-streaked dwarf virus in infected rice and their potential role in host gene regulation. Virol. J. 14, 27. https://doi.org/10.1186/s12985-017-0699-3

Yamaji, Y., Maejima, K., Ozeki, J., Komatsu, K., Shiraishi, T., Okano, Y., Himeno, M., Sugawara, K., Neriya, Y., Minato, N., Miura, C., Hashimoto, M., Namba, S., 2012. Lectin-mediated resistance impairs plant virus infection at the cellular level. Plant Cell 24, 778–93. https://doi.org/10.1105/tpc.111.093658

Yang, L., Li, B., Zheng, X.Y., Li, J., Yang, M., Dong, X., He, G., An, C., Deng, X.W., 2015. Salicylic acid biosynthesis is enhanced and contributes to increased biotrophic pathogen resistance in Arabidopsis hybrids. Nat. Commun. 6, 1–11. https://doi.org/10.1038/ncomms8309

Ye, J., Yang, J., Sun, Y., Zhao, P., Gao, S., Jung, C., Qu, J., Fang, R., Chua, N.H., 2015. Geminivirus Activates ASYMMETRIC LEAVES 2 to Accelerate Cytoplasmic DCP2-Mediated mRNA Turnover and Weakens RNA Silencing in Arabidopsis. PLoS Pathog. 11.

Ye, R., Wang, W., Iki, T., Liu, C., Wu, Y., Ishikawa, M., Zhou, X., Qi, Y., 2012. Cytoplasmic Assembly and Selective Nuclear Import of Arabidopsis ARGONAUTE4/siRNA Complexes. Mol. Cell 46, 859–870. https://doi.org/10.1016/j.molcel.2012.04.013

Yoshikawa, M., 2013. Biogenesis of trans-acting siRNAs, endogenous secondary siRNAs in plants. Genes Genet. Syst. 88, 77–84.

Yoshikawa, M., Iki, T., Tsutsui, Y., Miyashita, K., Poethig, R.S., Habu, Y., Ishikawa, M., 2013. 3′ fragment of miR173-programmed RISC-cleaved RNA is protected from degradation in a complex with RISC and SGS3. PNAS 110, 4117–4122. https://doi.org/10.1073/pnas.1217050110

Yu, X.D., Liu, Z.C., Huang, S.L., Chen, Z.Q., Sun, Y.W., Duan, P.F., Ma, Y.Z., Xia, L.Q., 2016. RNAi-mediated plant protection against aphids. Pest Manag. Sci.

Zhang, G., Chen, M., Li, L., Xu, Z., Chen, X., Guo, J., Ma, Y., 2009. Overexpression of the

soybean GmERF3 gene, an AP2/ERF type transcription factor for increased tolerances to salt, drought, and diseases in transgenic tobacco. J. Exp. Bot. 60, 3781–96. https://doi.org/10.1093/jxb/erp214

Zhang, H., Xia, R., Walbot, V., 2015. Evolution, functions, and mysteries of plant ARGONAUTE proteins. Curr. Opin. Plant Biol. 27, 84–90. https://doi.org/10.1016/J.PBI.2015.06.011

Zhang, W., Sun, X., Yuan, H., Araki, H., Wang, J., Tian, D., 2008. The pattern of insertion/deletion polymorphism in Arabidopsis thaliana. Mol. Genet. Genomics 280, 351–361. https://doi.org/10.1007/s00438-008-0370-1

Zhang, Xiaoming, Xia, J., Lii, Y., Barrera-Figueroa, B., Zhou, X., Gao, S., Lu, L., Niu, D., Liang, W., Chen, Z., Leung, C., Wong, T., Guo, J., Li, Y., Liu, R., Zhu, J.-K., Zhang, W., Jin, H., Zhang, H., 2012. Genome-wide analysis of plant nat-siRNAs reveals insights into their distribution, biogenesis and function. Genome Biol. 13, R20.

Zhang, X., Yuan, Y.-R., Pei, Y., Lin, S.-S., Tuschl, T., Patel, D.J., Chua, N.-H., 2006. Cucumber mosaic virus-encoded 2b suppressor inhibits Arabidopsis Argonaute1 cleavage activity to counter plant defense. Genes Dev. 20, 3255–68. https://doi.org/10.1101/gad.1495506

Zhang, Xiaofeng, Zhang, X., Singh, J., Li, D., Qu, F., 2012. Temperature-Dependent Survival of Turnip Crinkle Virus-Infected Arabidopsis Plants Relies on an RNA Silencing-Based Defense That Requires DCL2, AGO2, and HEN1. J. Virol. 86, 6847–6854. https://doi.org/10.1128/JVI.00497-12

Zhang, X., Zhao, H., Gao, S., Wang, W.-C., Katiyar-Agarwal, S., Huang, H.-D., Raikhel, N., Jin, H., 2011. Arabidopsis Argonaute 2 Regulates Innate Immunity via miRNA393∗-Mediated Silencing of a Golgi-Localized SNARE Gene, MEMB12. Mol. Cell 42, 356–366. https://doi.org/10.1016/j.molcel.2011.04.010

Zhang, Xiuchun, Zhang, Xiaofeng, Singh, J., Li, D., Qu, F., 2012. Temperature-dependent survival of Turnip crinkle virus-infected arabidopsis plants relies on an RNA silencing-based defense that requires dcl2, AGO2, and HEN1. J. Virol. 86, 6847–54. https://doi.org/10.1128/JVI.00497-12

Zhang, Z., Liu, X., Guo, X., Wang, X.-J., Zhang, X., 2016. Arabidopsis AGO3 predominantly recruits 24-nt small RNAs to regulate epigenetic silencing. Nat. Plants 2, 16049. https://doi.org/10.1038/nplants.2016.49

Zhao, K., Zhao, H., Chen, Z., Feng, L., Ren, J., Cai, R., Xiang, Y., 2015. The Dicer-like, Argonaute and RNA-dependent RNA polymerase gene families in Populus trichocarpa: gene structure, gene expression, phylogenetic analysis and evolution. J. Genet. 94, 317–321. https://doi.org/10.1007/s12041-015-0508-y

Zhao, Y., Chang, X., Qi, D., Dong, L., Wang, G., Fan, S., Jiang, L., Cheng, Q., Chen, X., Han, D., Xu, P., Zhang, S., 2017. A Novel Soybean ERF Transcription Factor, GmERF113, Increases Resistance to Phytophthora sojae Infection in Soybean. Front. Plant Sci. 8, 299. https://doi.org/10.3389/fpls.2017.00299

Zheng, X., Fahlgren, N., Abbasi, A., Berry, J.C., Carrington, J.C., 2019a. Antiviral ARGONAUTEs Against Turnip Crinkle Virus Revealed by Image-Based Trait Analysis. Plant Physiol. 180, 1418–1435. https://doi.org/10.1104/pp.19.00121

Zheng, X., Fahlgren, N., Abbasi, A., Berry, J.C., Carrington, J.C., 2019b. Antiviral ARGONAUTEs Against Turnip Crinkle Virus Revealed by Image-Based Trait Analysis. Plant Physiol. 180, 1418–1435. https://doi.org/10.1104/pp.19.00121

Zheng, X., Zhu, J., Kapoor, A., Zhu, J.-K., Liu, J., Chen, X., Harmer, S., Zilberman, D., Pikaard, C., Watson, J., Rezaian, A., Dennis, E., Waterhouse, P., Martienssen, R., 2007. Role of Arabidopsis AGO6 in siRNA accumulation, DNA methylation and transcriptional gene silencing. EMBO J. 26, 1691–701. https://doi.org/10.1038/sj.emboj.7601603

Zhou, X.-F., Sun, J.-D., Zhuo, Z., Jie, L. V, Wei, X.-W., Rui, C., Xu, H.-W., 2012. The Feasibility Analysis of PVX and TRV Vectors as the VIGS Tool for Studying the Gene Function peer-review under responsibility of [name organizer]. Int. Conf. Med. Phys. Biomed. Eng. 33, 46–54. https://doi.org/10.1016/j.phpro.2012.05.029

Zhou, Y., Honda, M., Duan, L., Zhang Correspondence, X., Zhu, H., Zhang, Z., Guo, X., Li, T., Li, Z., Peng, X., Nakajima, K., Zhang, X., 2015. Spatiotemporal Sequestration of miR165/166 by Arabidopsis Argonaute10 Promotes Shoot Apical Meristem Maintenance.

CellReports 10, 1819–1827. https://doi.org/10.1016/j.celrep.2015.02.047

Zhu, H., Hu, F., Wang, R., Zhou, X., Sze, S.-H., Liou, L.W., Barefoot, A., Dickman, M., Zhang, X., Qi, Y, Chen, S., Hannon, G., Qi, Y, Chen, X., Chen, X., Wu, Z., Qi, Yijun, Li, Y., 2011. Arabidopsis Argonaute10 Specifically Sequesters miR166/165 to Regulate Shoot Apical Meristem Development. Cell 145, 242–256. https://doi.org/10.1016/j.cell.2011.03.024

Zilberman, D., Cao, X., Jacobsen, S.E., 2003. ARGONAUTE4 control of locus-specific siRNA accumulation and DNA and histone methylation. Science 299, 716–9. https://doi.org/10.1126/science.1079695

Zust, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., Barchet, W., Diamond, M.S., Siddell, S.G., Ludewig, B., Thiel, V., 2011. Ribose 2'-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. Nat Immunol 12, 137–143.

2.10. Supplemental Data

2.10.1 Supplemental Figures.

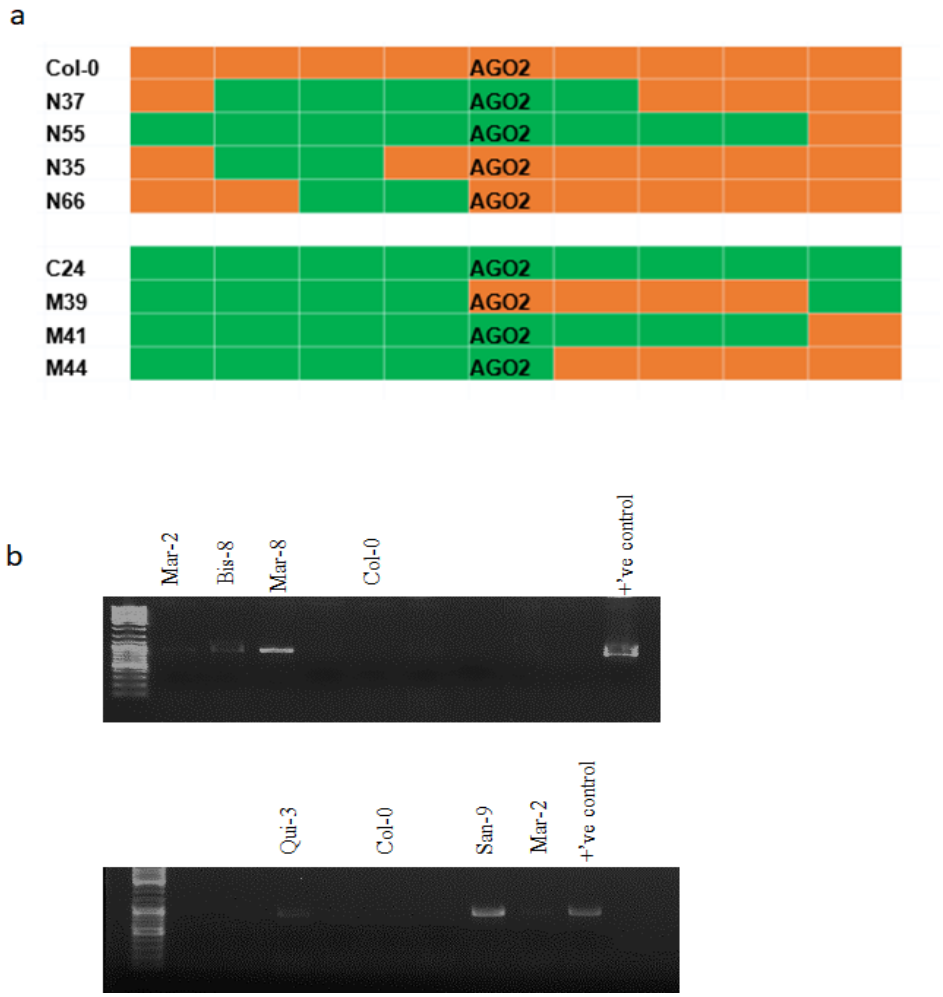


Figure S2.1. Origin of RILs and *JAX1* identity of Iberian Peninsula wild accessions. a, Schematic representation of chromosome 1 in recombinant RILs. Lines were generated from Col-0 and C24 parental plants (Törjék et al., 2008). N lines have a Col-0 background (orange) genome except for indicated segments of chromosome one exchanged with the corresponding region from C24 (green). M lines have a C24 background (green) with Col-0 substitutions (orange). b, Identification of functional *JAX1* by PCR on genomic DNA of Iberian Peninsula wild accessions with *JAX1* specific primers. Only *JAX1* positive lines and controls are indicated.

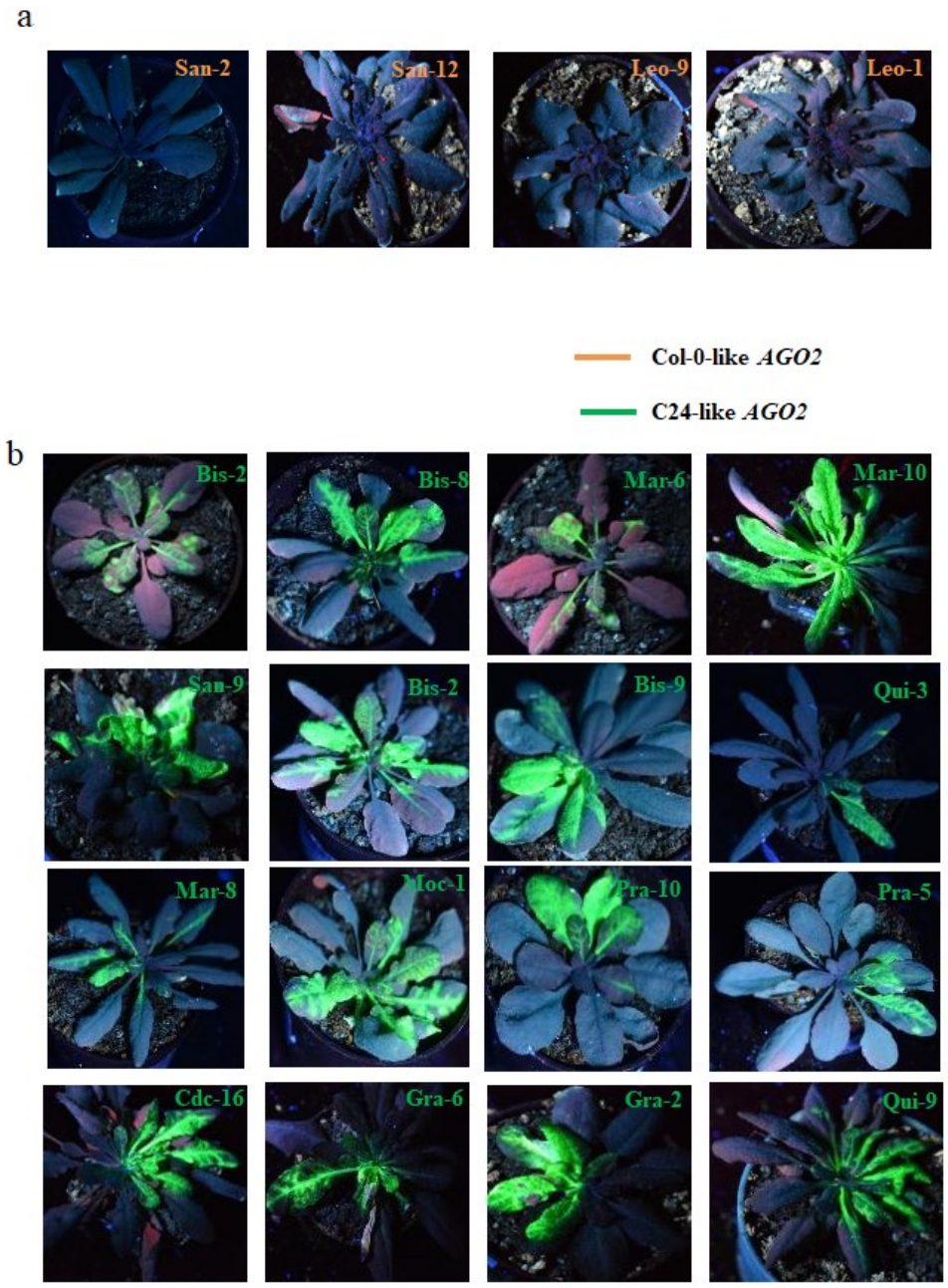


Figure S2.2. a, Infection of Iberian Peninsula wild accessions that possess a Col-0-like *AGO2* allele with TuMV-GFP-AS9. b, Infection of wild accessions that possess a C24-like *AGO2* with TuMV-GFP-AS9. Plants were photographed under hand-held UV illumination lamp at fifteen dpi.

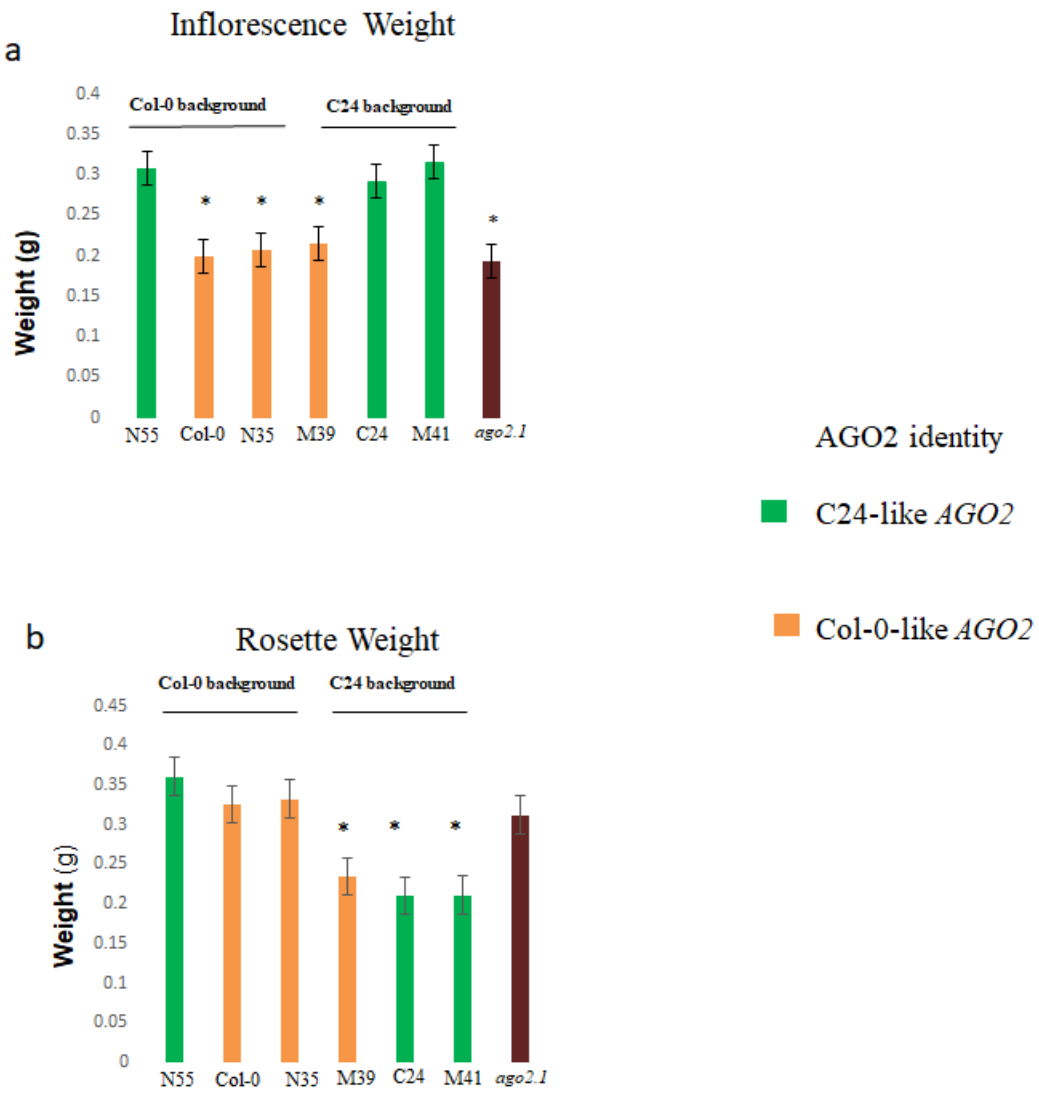


Figure S2.3. Life history traits of individual RILs. a, Inflorescence weight with seed of individual RILs, as indicated, as well as the *ago2.1* mutant. These lines are classified based on their *AGO2* allele identities and genomic backgrounds. b, Rosette weight of individual RILs and *ago2.1* mutant. These lines are classified based on their *AGO2* allele identities and backgrounds. Data are average values (g) with ±standard error bars of thirty plants per RILs. Asterisks indicate significant differences between lines ($P<0.05$).

Accession	*AGO2* allele	Allometric group
Bis-2	C24	2
Bis-8	C24	2
Gra-2	C24	1
Gra-6	C24	1
Leo-1	Col-0	1
Leo-11	Col-0	1
Leo-4	C24	1
Leo-9	Col-0	1
Moc-1	C24	2
Moc-9	Col-0	1
Pra-0	Col-0	1
Pra-10	C24	1
Pra-5	C24	2
Qui-3	C24	2
Qui-6	C24	1
Qui-9	C24	1
San-2	Col-0	1
San-4	Col-0	2
San-5	C24	2
San-9	C24	2

Table S2.1: Classification Iberian Peninsula wild accessions by allometric group and *AGO2* allele identity.

CHAPTER 3[2]

3. *Arabidopsis thaliana AGO2* confers tolerance and recovery to potato virus X in transgenic *Nicotiana benthamiana* and Tomato plants.

This article explores interspecific differences in the AGO2 protein of *Arabidopsis thaliana* and *Nicotiana benthamiana* plants. Having observed through transient expression assays that the *A. thaliana* AGO2 (AtAGO2), shows strong antiviral activity against potato virus X (PVX) but that the AGO2 protein of *Nicotiana benthamiana* (NbAGO2) does not, we set out to test if antiviral activity could be transferred between species by generating *AGO2* transgenic lines. Two constructs were generated; one expressing the coding sequence (CDS) of *AtAGO2* and the other, the CDS of *NbAGO2*, driven by the *NbAGO2* promoter and terminator. Both constructs were introduced into *N. benthamiana* and tomato plants. These transgenic plants were then inoculated with PVX and their response to virus infection was observed. Our result is the first to show that the introduction of an *A. thaliana* AGO protein into another model plant helps to confer tolerance to viral infection.

For work done in this article, A.A., C.B. and P.M., designed the experiments. A.A conducted the majority of initial work done in this study. C.B performed the characterization of the AGO2 protein in transgenic *N. benthamiana* lines and the effect of PVX on transgene expression as well as PVX and tomato experiments. A.A., and PM wrote the manuscript. The manuscript will continue to be improved as we hope to infect the transgenic plants with *Pepino mosaic virus* (PePMV) and investigate the effect of AtAGO2 on PepMV accumulation and *vice versa*.

[2] This chapter is yet to be published. It has been reformatted and reprinted from a submission to be made to a scientific journal once all experiments are completed: Ayooluwa Adurogbangba, Chantal Brosseau, and Peter Moffett. *Arabidopsis* AGO2 confers resistance to PVX in transgenic *Nicotiana benthamiana* and Tomato plants.

3.1 ABSTRACT

RNA silencing is a major mechanism of constitutive antiviral defense in plants. *A. thaliana* encodes four Dicer-like (DCL) proteins and ten different Argonaute (AGO) proteins that are specialized to function in different RNA silencing-related mechanisms. Several AGO proteins, including AGO2, have been shown to play important roles in protecting plants against viruses. We have previously shown that AGO2 precludes infection of *A. thaliana* Col-0 by potato virus X (PVX) in a manner reminiscent of non-host resistance (Jaubert et al., 2011). Likewise, transient expression assays have shown that the AGO2 protein from the PVX-susceptible host *Nicotiana benthamiana* (NbAGO2), is destabilized by PVX, whereas *A. thaliana* AGO2 (AtAGO2) is not (Brosseau et al., 2019), suggesting that PVX can counteract the silencing machinery in its host, but not in Arabidopsis. To test whether virus resistance could be transferred between plants, we introduced constructs expressing NbAGO2 and AtAGO2 into *N. benthamiana* and tomato plants. We show that *N. benthamiana* and tomato plants expressing AtAGO2 are more tolerant to PVX infection when compared to plants expressing NbAGO2 construct or non-transgenic plants. These results demonstrate that RNA silencing-mediated non-host resistance can be transferred from one plant species to another.

Keywords: RNA silencing, virus defense, non-host resistance, AGO2, PVX, Transgenic *N. benthamiana*, tomato,

3.2 INTRODUCTION

RNA silencing plays important roles in plant development and genome stability in most eukaryotes (Carbonell and Carrington, 2015). In higher plants, RNA silencing is also a posttranscriptional regulatory process that functions as an antiviral defense mechanism. It relies on virus-derived small RNAs (sRNAs) ranging from 21 to 24 nucleotides (nts) (Dunoyer et al., 2010; Dzianott et al., 2012b; Molnar et al., 2010). The presence of double-stranded RNA (dsRNA) replication intermediates of viruses or intramolecular fold-back structures within their genomes triggers antiviral RNA silencing. These dsRNAs are processed by ribonuclease III-like enzymes, called Dicer-like proteins (DCLs) to generate short interfering RNA (siRNA) (Zhao et al., 2015). The siRNAs bind to plant Argonaute (AGO) proteins, a component of the RNA-induced silencing complexes (RISC), and guide the AGO protein to target viral or host RNA. The AGO protein anneals to the target RNA through base-pairing and then cleaves the target RNA, leading to gene silencing (Carbonell and Carrington, 2015).

To overcome the antiviral defense of RNA silencing, plant viruses encode viral silencing suppressors (VSRs) as a counter-defense strategy and mechanism. Many VSRs have been reported to inhibit different aspects of RNA silencing mechanisms. For example, the P19 protein of tomato bushy stunt virus (TBSV) has been reported to sequester siRNA (Kontra et al., 2016). Of the ten AGO proteins encoded by *A. thaliana,* the requirement of AGO2 protein for antiviral defense against several virus infections has been shown (Carbonell and Carrington, 2015). In addition, in *N. benthamiana* plants, AGO2 is involved in defense against a number of viruses (Fátyol et al., 2016; Ludman et al., 2017a; Odokonyero et al., 2015; Scholthof et al., 2011). However, despite having an AGO2 protein, *N. benthamiana* plants are susceptible to viruses, including PVX (Brosseau et al., 2019).

To limit or avoid infections from pathogens, host plants employ a number of defense mechanisms leading to resistance, tolerance or recovery. Tolerance is a well-documented phenomenon in which viruses may accumulate to some degree within the plant without causing significant loss of yield or fitness to the plant (Pagán and García-Arenal, 2018). Indeed, tolerance may be defined as the

ability of the host to limit the effect(s) of infection on its fitness regardless of the level of multiplication of the pathogen (Paudel and Sanfaçon, 2018). Symptom recovery in plants infected with viruses is another well-studied field that has shown scenarios where the virus remains in the host cell at low levels and has no detrimental effect on the host (Ghoshal and Sanfaçon, 2014; Matić et al., 2019; Priya Raja et al., 2008; Saumet and Lecellier, 2006). RNA silencing has been reported to be essential for viral symptoms recovery in some plants (Bengyella et al., 2015; Eamens et al., 2008; Ma et al., 2015), and NbAGO1 is essential for recovery to Tomato ringspot virus (ToRSV) in *N. benthamiana* (Ghoshal and Sanfacon 2014). Furthermore, RNA silencing has been reported to act as a non-host resistance (Jaubert et al., 2011). Non-host resistance is a type of immunity that is present in an entire plant species to all genetic variants of a non-adapted pathogen species (Lee et al., 2016). Therefore, non-host resistance has been considered a valuable and practical tool for crop protection (Gill et al., 2015; Lee et al., 2013; Senthil-Kumar and Mysore, 2013). However, most studies that have aimed to introduce non-host resistance in crop species have introduced genes that provide protection against fungal and bacterial pathogens (Lee et al., 2016). In contrast, by exploiting components of the RNA silencing pathway, such as AGO proteins, crop plants can be engineered for non-host tolerance to viruses.

PVX is a virus of economic importance and it has often been used as a model to study plant-virus interactions in order to elucidate plant defense mechanisms (Zhou et al., 2012). The main hosts of PVX are herbaceous plants, especially *Solanaceae*, mainly potatoes and tomato (Lico et al., 2015). PVX infection is latent and it causes mosaic symptoms (Lico et al., 2015). In its natural host, symptoms are variable, and depend on the PVX strain (Lico et al., 2015). In potato plants, the importance of PVX infections to the yield of crops has been mitigated through seed certification programs (Frost et al., 2013; Thomas-Sharma et al., 2016). However, in tomato plants, PVX infection causes reduction in growth and photosynthesis, and the alteration of other physiological characteristics (Balogun, 2003). *A. thaliana* Col-0 is a non-host for PVX, but when *AGO2* is mutated, *A. thaliana,* becomes susceptible to PVX (Jaubert et al., 2011).

Previous work has also shown that the AGO2 and AGO5 proteins are responsible for restricting PVX infection in *A. thaliana* in a spatial/temporal cooperative fashion (Brosseau and Moffett,

2015). The co-expression by *A. tumefaciens*-mediated infiltration of AGO2 proteins from *A. thaliana* and *N. benthamiana* in *N. benthamiana* leaves in the presence of PVX and PVXΔTGB (which lacks the VSR, P25) revealed that while the AGO2 protein from *A. thaliana* was able to suppress the accumulation of PVX, NbAGO2 was not. Both AGO2 proteins, however, repressed the accumulation of PVXΔTGB (Brosseau et al., 2019). This result suggests that both AGO2 proteins have the intrinsic ability to target viral RNAs, but that PVX is able to overcome the activity of NbAGO2. Indeed, NbAGO2 is targeted/degraded by PVX, whereas AtAGO2 is not (Brosseau et al., 2019), explaining why NbAGO2 has little activity against PVX. The degradation of NbAGO2 was dependent on the presence of P25 as the co-expression of PVXΔTGB does not affect the levels of NbAGO2 protein expression. Based on these findings, we aimed to transfer the non-host resistance against PVX from *A. thaliana* to *N. benthamiana* plants by generating transgenic *N. benthamiana* plants, expressing Col-0 AtAGO2 (AtAGO2) and NbAGO2. With these transgenic plants, we hypothesized that AtAGO2 and not NbAGO2, will help confer tolerance in *N. benthamiana* plants during PVX infection. We showed that AtAGO2 protein, when introduced into *N. benthamiana* and tomato plants, restricts the systemic movement of PVX infection in both plant species. In addition, these transgenic AtAGO2 plants have fewer viral symptoms when compared to the NbAGO2 transgenic and wild-type plants. Our results are novel as we show that non-host resistance can be introduced from one plant species to crops. Indeed, work done in this study may serve as a model for the generation of transgenic virus-tolerant plants.

3.3 MATERIALS AND METHODS

3.3.1 Generation and selection of transgenic plants.

Agrobacteria tumefaciens -mediated stable transformation of *N. benthamiana* and tomato plants were performed as previously described in the literature. *A. tumefaciens* expressing the desired construct (NbAGO2 or Col-0 AtAGO2) were grown and re-suspended to final OD_{600} = 0.5. Two to four *N. benthamiana* or tomato leaves were submerged in the suspension. The leaves were then placed on the adaxial side (upper side) on the shoot inducing medium for co-cultivation. Plates were covered with aluminum foil and co-cultivated for 72 hours or until the generation of somatic

cells grow from the abaxial side. After three weeks, the regenerated shoots were transferred to rooting media and rooted plants were transferred to soil. Both *N. benthamiana* and tomato plants with the desired transgenes were selected by performing a PCR reaction. To genotype *AGO2* alleles, genomic plant DNA was extracted using DNA extraction buffer (50mM NaCl, 100mM tris-HCl pH 8.0, 50mM EDTA pH 8.0) using a phenol-chloroform extraction method. DNA pellets were re-suspended in ddH_2O at a concentration of 20-100ng and then used as template for the PCR reaction. Each PCR reaction contained ThermoPol Buffer 10X, Taq DNA Polymerase (New England Biolabs), 0.2mM of dNTP mix, 1.5mM of $MgCl_2$, 0.6 µM of specific primers either for AtAGO2 or NbAGO2. For NbAGO2, forward primer: (NbAtAGO2: 5'-GCCACCATGTACCCATACGATGG-3'), reverse primer: (NbAGO2R: 5'-TCTGAGCTATTGGGTTGCACATCC) and AtAGO2 detection (same forward primer as NbAtAGO2) with a reverse primer sequence of (AtAGO2R5'-CTCTATCAGGTCGCTTCATTGGTTCTT-3'). PCR mixtures for *AGO2* detection were incubated for 2 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 40 s, and PCR products were analyzed by electrophoresis on 2% agarose

3.3.2 Plant material and growth conditions and virus inoculation.

N. benthamiana wild type and transgenic plants were grown on soil (BM6) in growth chambers with 16-h-light/8-h-dark photoperiod at 23°C. Virus inoculation of three-week-old *Nicotiana benthamiana* wild type, tomato wild type and transgenic plants were carried out by rub inoculation as previously described (Jaubert *et al*., 2011). Three leaves per plant were rub inoculated. Viral saps were produced with PlAMV-GFP or PVX-GFP-infected *N. benthamiana* plants by grinding infected tissue in 0.1M phosphate buffer, pH 7.0 (2 mL/g of infected tissues). Mock infections were carried out with sap produced with uninfected *N. benthamiana* plants (2 mL/g of uninfected tissues). Viral assessment was carried out at seven, nine, fifteen- and twenty-one-days post inoculation.

3.3.3 Plasmid Construction

For the generation of pNbAGO2::NbAGO2::NbAGO2Term (NbAGO2) and pNbAGO2::AtAGO2::NbAGO2Term (AtAGO2) constructs, Col-0 AGO2 upstream (2068 bp) and downstream (1034 bp) regulatory sequences were amplified from genomic DNA with primers listed in Supplementary Table 1. PCR products were purified, cloned into the pGEM-T easy vector (Promega) and subcloned into the Acc65I/XbaI or BamHI/SbfI sites of pBIN61 vector to replace the 35S promoter and terminator respectively. AGO2 coding sequences were amplified from cDNA with primers listed in Supplementary Table 3.1, cloned into the pGEM-T easy vector (Promega) and subcloned into the XbaI/BamHI sites of pNbAGO2::NbAGO2Term. PVX, PVX-GFP and PVX-GFPΔTGB binary constructs (Bhattacharjee et al., 2009; Peart et al., 2002b) have been previously described.

3.3.4 Transient Expression Assays

Agrobacterium tumefaciens-mediated transient expression assays in *N. benthamiana* were performed as previously described (Moffett, 2011). Briefly, binary expression constructs were transformed into the C58C1 *Agrobacterium tumefaciens* strain carrying pCH32 virulence plasmid. For virus agro-infiltration, GV3101 *Agrobacterium tumefaciens* strain carrying the pSoup helper plasmid was transformed with pGr106/pGr107/pGr208 derivatives constructs (PVX-GFP, PVX-GFP-ΔTBG). *Agrobacterium tumefaciens* cultures were centrifuged at 4,000 RPM for 10 min and re-suspended in 10 mM $MgCl_2$ to a final OD_{600} = 0.1 for virus vectors.

3.3.5 Protein Extraction and Analysis

1 g of fresh tissues was ground into 2 mL of RISC buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 5 mM MgCl2, 5 mM dithiothreitol [DTT], 0,5% NP-40) supplemented with protease inhibitor cocktails. Total protein extracts were centrifuged at 16 000 xg for ten minutes at 4°C. A fraction of total protein extract was kept for detection of GFP. Immunoprecipitation was carried out with 1.4 ml of supernatant and 25 µl of HA-agarose beads (Sigma) for 2 h at 4°C on a rotatory shaker. Beads were washed 4 times with RISC buffer. After centrifugation, beads were re-

suspended in 50 µl of 1.5X of Laemmli loading buffer (Laemmli, 1970). Proteins were separated by SDS-PAGE on 7.5% or 10.5% acrylamide gels for AGO or GFP, and CP detection, respectively, and transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad) by electro-blotting. HA-AGO proteins were detected with anti-HA-horseradish peroxidase conjugated (HRP) antibodies (Sigma, 1:3,000 dilution). Detection of GFP was carried out by probing membranes with anti-GFP-HRP antibodies (Santa Cruz, 1:3,000 dilution). Anti-PVX-CP rabbit polyclonal antibodies (Agdia, 1:3,000 dilution) was used to detect PVX-CP in these transgenic plants, followed by donkey anti-IgG rabbit-HRP polyclonal antibodies (BioLegend, 1:10,000 dilution). Detection of HA: AGO2 protein was carried out by probing membranes with anti-HA antibody (Agrisera, 1:3,000 dilution) and subsequently with donkey anti-IgG rabbit-HRP polyclonal antibodies (BioLegend, 1:10,000 dilution). Equal loading of proteins was confirmed with ponceau.

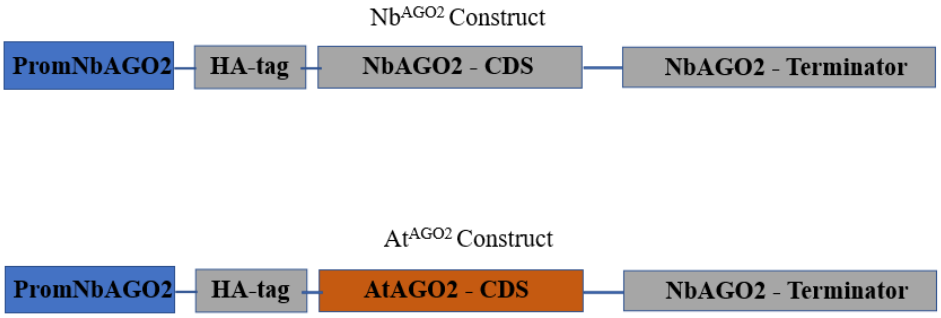
3.4 RESULTS

3.4.1 Generation of transgenic *N. benthamiana* and tomato plants.

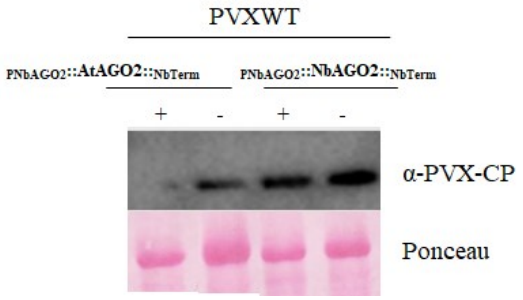
Previous results indicated that AtAGO2 functions more effectively against PVX than NbAGO2 (Brosseau et al., 2019), strongly suggesting that AGO2 can act as a host range factor. However, these experiments were undertaken using strong transient over expression. We therefore sought to test if the transfer of Col-0 *AGO2* (*AtAGO2*) from *A. thaliana* to another species can result in the generation of virus-resistant transgenic plants. To do so, expression cassettes were generated containing either the Col-0 *A. thaliana* or the *N. benthamiana AGO2* coding sequence (CDS) driven by the *NbAGO2* genomic promoter and terminator sequences [P_{NbAGO2}::AtAGO2::NbAGO2Term (AtAGO2); P_{NbAGO2}::NbAGO2::NbAGO2Term (NbAGO2)] (Figure 3.1A). Both constructs were tested in *Agrobacteria*-mediated transient expression (agroinfiltration) assays in *N. benthamiana* leaves, together with PVX. PVX accumulation, as determined by anti-CP immune blotting, was undetectable in the presence of AtAGO2, while levels of PVW-WT-GFP remained unchanged in the presence of NbAGO2 construct (Figure 3.1B). In addition, we expressed PVXΔTGB-GFP in the absence or presence of both genomic

constructs (Figure 3.1C). In the presence of the AtAGO2 construct, no accumulation of PVX-CP was detected by immunoblotting. Likewise, when NbAGO2 was co-expressed with PVXΔTGB, a reduction in the accumulation of PVX was observed (Figure 3.1C). Although the reduction of the latter by NbAGO2 was less dramatic than AtAGO2, this nonetheless indicates that the protein still retains some function.

A



B



C

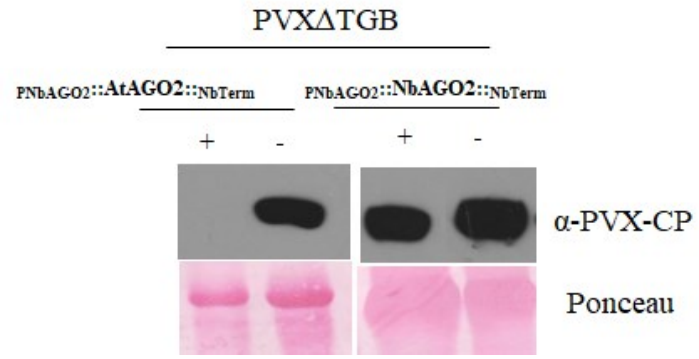


Figure 3.1. Testing of AGO2 genomic clones using PVXΔTGB and PVX-WT. A) Schematic diagram of the P_{NbAGO2}::NbAGO2::NbAGO2Term (NbAGO2) and P_{NbAG02}::AtAGO2::NbAGO2Term constructs (AtAGO2). **B)** PVX were transiently co-expressed by agroinfiltration in *N. benthamiana* with or without AtAGO2 and NbAGO2 clones. Three days later, protein extraction was performed, and samples were subjected to immune blot analysis detecting accumulation levels of PVX, through anti-PVX-CP immunoblotting. Ponceau staining (bottom panel) of the same extracts is shown to indicate equal loading. **C)** PVXΔTGB-GFP were transiently co-expressed by agroinfiltration in *N. benthamiana* with or without AtAGO2 and NbAGO2 clones. Three days later, protein extraction was performed, and samples were subjected to immune blot analysis detecting accumulation levels of PVXΔTGB protein through anti-PVX-CP antibody.

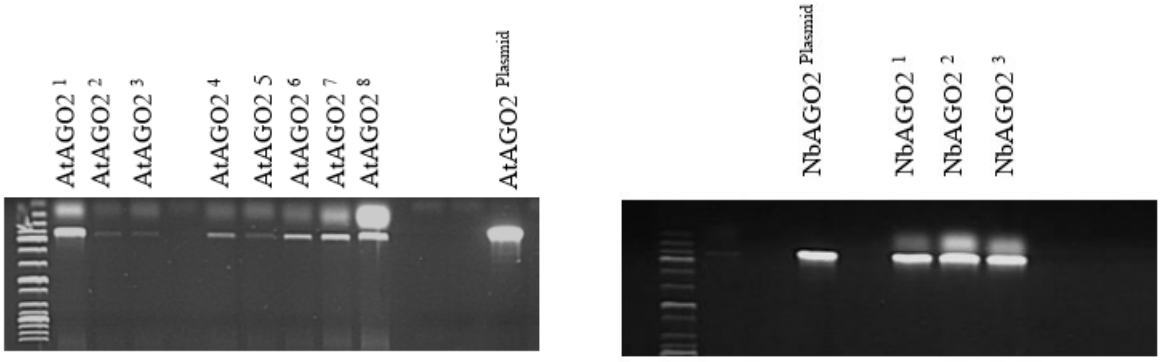
3.4.2 Transgenic plants expressing AtAGO2 are less susceptible to PVX.

The transgenic plants were selected by performing PCR to detect either NbAGO2 or AtAGO2 (as previously described) prior to infection (Figure 3.2A). In addition, in order to ensure that the introduced AGO2 proteins (AtAGO2 and NbAGO2) were expressed in these transgenic plants, immune blotting was performed, using HA antibody to detect the exogenous AGO2 protein (Supplementary Figure 1). Wild-type *N. benthamiana* plants, as well as transgenic lines expressing both AtAGO2 and NbAGO2 transgenes, were inoculated with PVX-GFP (Figure 3.2B and C). At fifteen dpi, the systemic movement of PVX, as well as the disease, were monitored visually by observing the expression of GPF in upper non-inoculated leaves with a hand-help UV lamp (Figure

3.2B). The NbAGO2 plants possessed more virus symptoms when compared to their AtAGO2 counterpart but less symptoms compared to wild type plants. Also, PVX movement was restricted in the AtAGO2 plants as less symptoms and viral accumulation were observed in upper non-inoculated leaves (Figure 3.2B-E).

In addition, levels of accumulation of PVX CP was detected in upper non-inoculated tissues by immune blotting. We observed that the levels of accumulation of PVX in all the plants were similar at ten-dpi. (Figure 3.2D). However, twenty-one dpi, all the plants expressing the AtAGO2 construct showed significant decrease in the accumulation of PVX in upper non-inoculated tissue when compared to plants expressing the NbAGO2 construct and wild-type plants (Figure 3.2E). Together, these results indicate that the AtAGO2 confers recovery to PVX when introduced into *N. benthamiana.*

A



B


Nb^AGO2 N. benthamiana plants
At^AGO2 N. benthamiana plants

C


I
II
III
IV
WT uninfected
WT infected
At^AGO2 infected
Nb^AGO2 infected

D

E

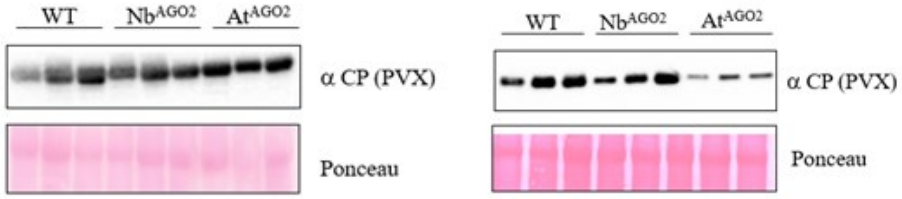


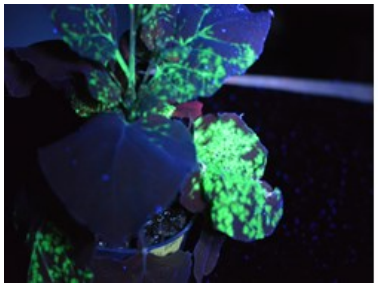
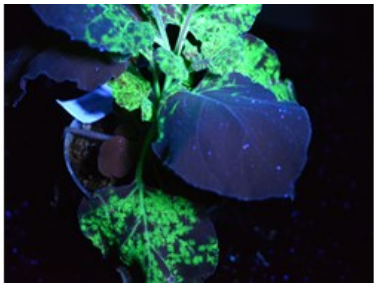
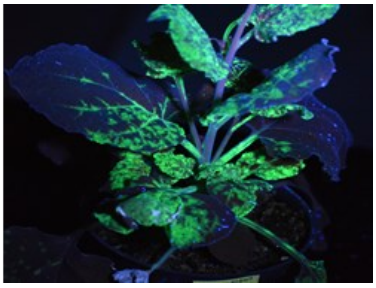
Figure 3.2. AtAGO transgenic plants showed reduced viral symptoms and viral accumulation in upper non-inoculated leaves. A) PCR was performed with transgenic plants and controls. Eight independent AtAGO2 positive lines were identified while three NbAGO2 independent positive lines were identified. Plasmid DNA of each construct were used as positive controls. **B)** PVX, expressing GFP was rub inoculated on transgenic lines expressing either AtAGO2 construct or NbAGO2 construct and virus movement was monitored. Pictures were taken fourteen days post inoculation of the PVX virus. GFP was visualized using a hand-held UV lamp while bright field images show levels of symptoms in both transgenic lines. **C)** Individual upper non-inoculated leaves were harvested from plants at fifteen dpi. Leaves from wild type plant uninfected plant was used as a negative control (I), wild type infected plant (II) as positive control, AtAGO2 infected (III) and NbAGO2 infected (IV). **D)** PVX-GFP was inoculated onto transgenic plants expressing either AtAGO2 or NbAGO2 constructs. Upper non-inoculated leaves were harvested at ten dpi and and samples were subjected to western blot analysis to detect the accumulation levels of PVX coat protein (CP) as indicated. **E)** Upper non-inoculated plant leaves were harvested again at twenty-one dpi subjected to western blot analysis detecting accumulation levels of PVX coat protein (CP) as indicated. Ponceau staining (bottom panel) of the same extracts is shown to indicate equal loading. Five plants were sampled in each experiment and all experiments were repeated at least three times.

3.4.3 AtAGO2 does not confer tolerance to PlAMV.

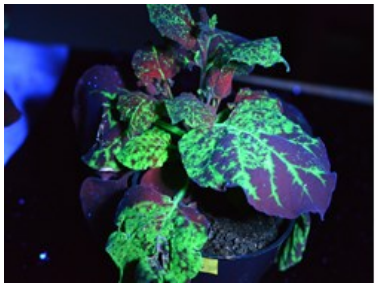
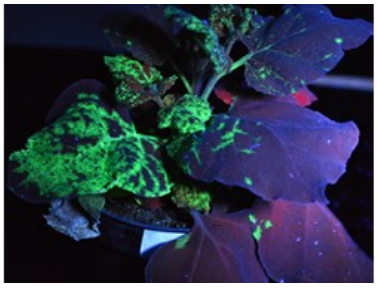
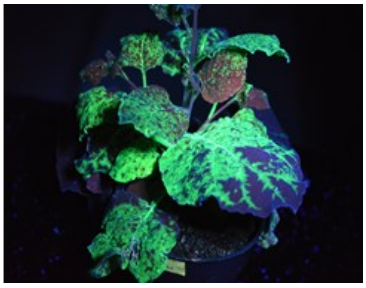
Having shown that the AtAGO2 plants were more tolerant to PVX-GFP, we investigated if this tolerance is observed with PlAMV, a virus that has been reported to overcome the antiviral activities of AtAGO2 (Brosseau et al., 2016). WT, transgenic AtAGO2 and NbAGO2 plants were rub inoculated with PlAMV-GFP and viral movement was monitored visually. In the presence of both AtAGO2 and NbAGO2, the accumulation of PlAMV was not compromised. At fourteen dpi,

PlAMV was present in upper non-inoculated leaves and tissue in wild type and transgenic plants. In addition, there was no difference in the accumulation PlAMV in all plants (Figure 3.3). Therefore, AtAGO2 transgenic plants are only tolerant to viruses that overcome AtAGO2 in nature.

WT

AtAGO2

NbAGO2

Figure 3.3 WT and transgenic plants are susceptible to PlAMV. Wild type, AtAGO2 and NbAGO2 transgenic plants were inoculated with PlAMV expressing GFP. Virus movement was monitored. Plants were photographed under UV illumination, using a hand-help UV lamp at fourteen dpi. Three plants per genotype were tested in each experiment and experiments repeated three times

3.4.4 In tomato, AtAGO2 delays viral movement and confers tolerance to PVX.

Wild-type tomato plants, as well as transgenic lines expressing both AtAGO2 and NbAGO2 transgenes, were inoculated with PVX-GFP (Figure 3.4). At ten dpi, the systemic movement of PVX, as well as the disease, were monitored visually by observing the expression of GPF in upper non-inoculated leaves with a hand-help UV lamp (Figure 3.4A). The NbAGO2 plants possessed more virus symptoms when compared to their AtAGO2 counterpart and similar levels compared to wild type plants. In addition, levels of accumulation of PVX CP was detected in upper non-inoculated tissues by immune blotting. In infected leaves, at ten dpi, the levels of accumulation of PVX in all the plants were similar. In upper non-inoculated leaves however, at ten dpi, the AtAGO2 tomato plants showed no accumulation of PVX in upper non-inoculated tissue, while the NbAGO2 plants showed similar PVX accumulation as wild-type plants (Figure 3.4D). These results indicate that the AtAGO2 confers resistance to PVX when introduced into tomato. Also, the AGO2 levels in these transgenic plants were assessed (Figure 3.4B and D). We observed that in infected and upper non-inoculated leaves, there was a noticeable increase in the accumulation of NbAGO2 while AtAGO2 levels remained similar (Figure 3.4B and D).

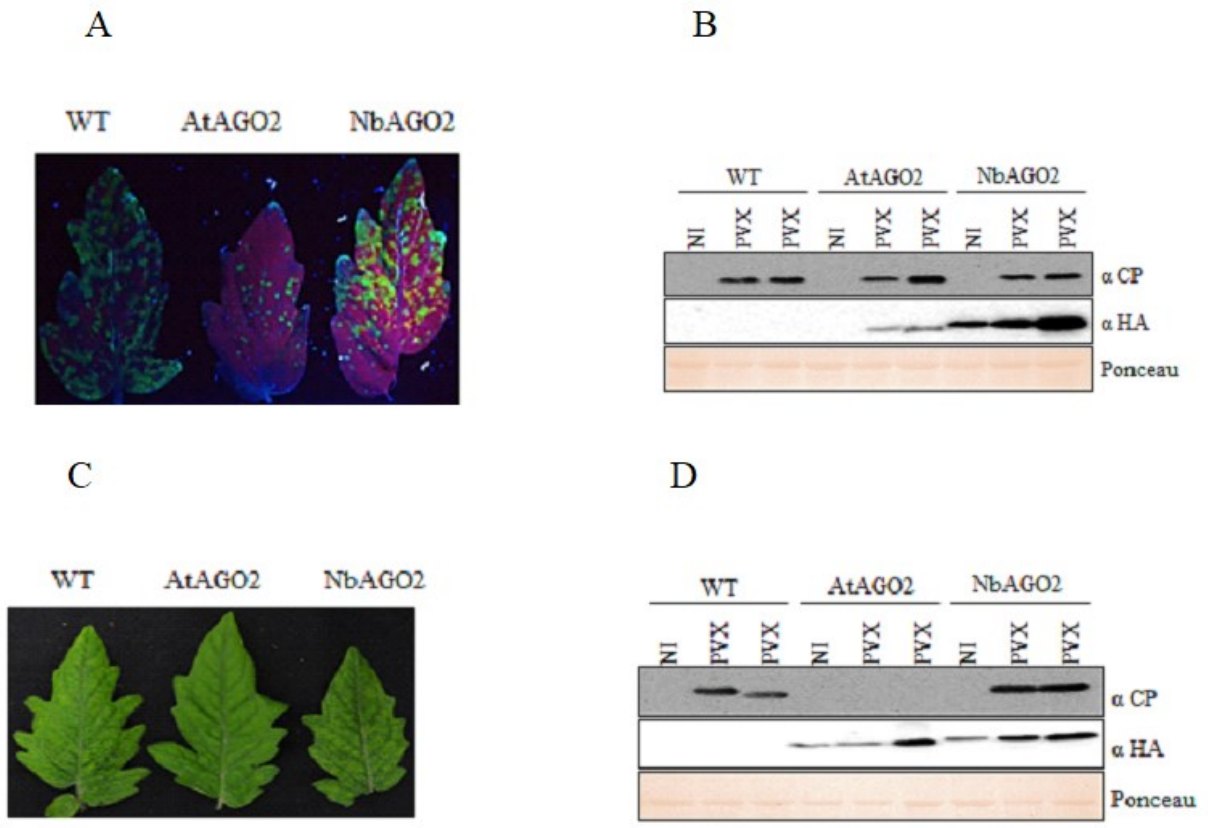


Figure 3.4 AtAGO2 delays PVX movement and confers resistance to PVX in tomato plants. **A)** PVX-GFP was rub inoculated on transgenic lines expressing either AtAGO2 or NbAGO2 as well as non-transgenic (WT) plants, as indicated, and virus movement was monitored. Pictures were taken ten dpi. GFP was visualized using a hand-held UV lamp. B). Inoculated leaves were harvested again at ten dpi and subjected to western blot analysis detecting accumulation levels of PVX coat protein (CP) and AGO levels (αHA) as indicated. **C)** Individual upper non-inoculated leaves were harvested from plants PVX-GFP inoculated plants at ten dpi. Leaves from wild type infected plant were used as positive control, AtAGO2 and NbAGO2 transgenic tomato plants were infected as well. **D)** Upper non-inoculated leaves were harvested at ten dpi and samples were subjected to immune blot analysis with αPVX coat protein (CP) and αHA (AGO2) antibodies as indicated as indicated. Ponceau staining (bottom panel) of the same extracts is shown to indicate equal loading. Five plants were sampled in each experiment.

3.5 DISCUSSION

It has been reported that NbAGO2 plays some role in susceptibility to PVX, as *ago2* knock-out (KO) *N. benthamiana* plants are susceptible to PVX infection compared to WT Col-0 plants (Ludman et al., 2017). Here, we have exploited the inter-species differences in the AGO2 protein by introducing Col-0 AtAGO2 into *N. benthamiana* and tomato plants to generate resistance to PVX. Our results show that in the presence of AtAGO2, *N. benthamiana* plants recover from PVX infections to a much greater degree than the *N. benthamiana* expressing NbAGO2 or WT plants, as judged by a decrease in the accumulation of PVX-CP in upper non-inoculated leaves at twenty-one dpi (Figures 3.2). At the same time, AtAGO2 appears to confer resistance to PVX in tomato (Figure 3.4). We propose that these differing results are due to differing levels of susceptibility to PVX. In *A. thaliana* Col-0, there is a weak initial accumulation of PVX in inoculated leaves, but the virus is undetectable after sometime (Brosseau et al., 2019). In *ago2* mutant *A. thaliana* plants, although as a lower level than in WT tomato, PVX accumulates in upper non-inoculated tissues and much lower than in *N. benthamiana* (Jaubert et al., 2011). As such, there is spectrum of responses to PVX, from hyper-susceptible infection in *N. benthamiana ago2* KO plants (Ludman et al., 2017), mild recovery in WT *N. benthaminana*, increased recovery in AtAGO2 transgenic *N. benthamiana*, complete systemic resistance in AtAGO2 transgenic tomato and *A. thaliana* Col-0. These responses are inversely correlated with the baseline susceptibility of these plants, suggesting that there is a threshold of virus accumulation at which AGO2 proteins can be effective.

We show that *AtAGO2* transgenic *N. benthamiana* plants initially displayed high levels of PVX accumulation in inoculated leaves and that the most dramatic effects (increased recovery or resistance) were seen in upper non-inoculated tissues (Figure 3.2). This raises questions regarding the mode and location of AGO2 action. For example, a certain threshold of viral RNA or vsiRNAs may need to be met before AGO2 is effective. Alternatively, AGO2 may have a greater effect on systemic movement of viruses, rather than acting primarily in inoculated leaves, although the latter would be in disagreement with previous work in *A. thaliana* (Brosseau and Moffett, 2015). Further research will be required to shed light on these issues. However, our observations suggest that *AtAGO2* transgenic plants can be either resistant (tomato) or tolerant (*N. benthamiana*) to PVX.

Tolerance when compared to resistance has been reported to have potential benefits to the hosts (Best et al., 2008). In nature, tolerance can be as good as resistance, since tolerance has been reported to cause no significant loss of yield or fitness to the plant (Pagan and Garcia-Arenal, 2018). Indeed, there are some examples of plants that have been engineered for tolerance to viral infections. The overexpression of the soybean transcription factor, GmERF3 gene in tobacco has been reported to confer increased tolerance to tobacco mosaic virus (Zhang et al., 2009; Zhao et al., 2017). In addition, over expression of NtRFP1 RING E3 ligase *N. benthamiana* plants promoted tolerance to a *begomovirus* (Shen et al., 2016). However, virus tolerance can result in the buildup of virus reservoirs, and it is therefore desirable to aim for resistance instead. Since AGO2 effectiveness is determined by the degree of host susceptibility, we propose that transferring non-host AGO2 proteins between plants may be effective in certain cases, but in others, should be accompanied by additional strategies to reduce overall susceptibility.

The transgenic plants were inoculated with PlAMV to determine if viral resistance or tolerance is achieved in AtAGO2 transgenic plants for all viruses. In contrast to the results seen with PVX, AtAGO2 transgenic *N. benthamiana* plants showed high accumulation of PlAMV in upper non-inoculated leaves and did not recover from PlAMV infection, (Figure 3.3). However, this finding is not surprising as PlAMV infects the *ago2* mutant in *A. thaliana* only slightly better than WT plants (Brosseau et al., 2016). Thus, PlAMV can overcome the antiviral effects of AtAGO2 presumably through the action of its very effective VSR (Brosseau et al., 2016) and so providing the *N. benthamiana* with *AtAGO2* does not increase resistance to this virus. This result highlights possible limitations to using heterologous AGO2 proteins to engineer resistance. Doing so would require an AGO2 protein that cannot be counteracted by the VSR of the virus in question. Likewise, this approach may not be effective against viruses whose VSR strategy (such as vsiRNA sequestration) does not involve inhibiting AGO proteins.

In summary, our work is the first to generate virus tolerant/resistant plants by transferring a more effective RNA silencing component from one plant to another. Our proof of principle opens a potentially new avenue for generating virus resistance plants. That is, in theory, for viruses that normally counteract AGO2 function, it may suffice to introduce an AGO2 protein from a plant

with which it has not co-evolved and thus unable to inhibit. Ultimately, it will also be of interest to determine why viruses such as PVX can inhibit certain AGO2 proteins (i.e. NbAGO2) but not others (i.e. AtAGO). An understanding of these mechanisms could result in the ability to introduce only small changes in the native AGO2 by genome editing to make it more effective against viruses.

3.6 References

Adams, M.J., Antoniw, J.F., Fauquet, C.M., 2005. Molecular criteria for genus and species discrimination within the family Potyviridae. Arch. Virol. 150, 459–479. https://doi.org/10.1007/s00705-004-0440-6

Adenot, X., Elmayan, T., Lauressergues, D., Boutet, S., Bouché, N., Gasciolli, V., Vaucheret, H., 2006. DRB4-Dependent TAS3 trans-Acting siRNAs Control Leaf Morphology through AGO7. Curr. Biol. 16, 927–932. https://doi.org/10.1016/j.cub.2006.03.035

Agorio, A., Vera, P., 2007. ARGONAUTE4 is required for resistance to Pseudomonas syringae in Arabidopsis. Plant Cell 19, 3778–90. https://doi.org/10.1105/tpc.107.054494

Akbergenov, R., Si-Ammour, A., Blevins, T., Amin, I., Kutter, C., Vanderschuren, H., Zhang, P., Gruissem, W., Meins, F., Hohn, T., Pooggin, M.M., 2006. Molecular characterization of geminivirus-derived small RNAs in different plant species. Nucleic Acids Res. 34, 462–471. https://doi.org/10.1093/nar/gkj447

Alazem, M., He, M.-H., Moffett, P., Lin, N.-S., 2017. Abscisic Acid Induces Resistance against Bamboo Mosaic Virus through Argonaute2 and 3. Plant Physiol. 174, 339–355. https://doi.org/10.1104/pp.16.00015

Anandalakshmi, R., Marathe, R., Ge, X., Herr, J.M., Mau, C., Mallory, A., Pruss, G., Bowman, L., Vance, V.B., 2000. A Calmodulin-Related Protein That Suppresses Posttranscriptional Gene Silencing in Plants. Science (80-.). 290.

Andika, I.B., Maruyama, K., Sun, L., Kondo, H., Tamada, T., Suzuki, N., 2015. Differential contributions of plant Dicer-like proteins to antiviral defences against potato virus X in leaves and roots. Plant J. 81, 781–793. https://doi.org/10.1111/tpj.12770

Ange´lique Deleris, Javier Gallego-Bartolome, Jinsong Bao, Kristin D. Kasschau, James C. Carrington, O.V., 2006. Hierarchical Action and Inhibition of Plant Dicer-Like Proteins in Antiviral Defense. Science (80-.). 305, 1462–322. https://doi.org/10.1126/science.1128650

Axtell, M.J., 2013. Classification and Comparison of Small RNAs from Plants. Annu. Rev. Plant Biol. 64, 137–159. https://doi.org/10.1146/annurev-arplant-050312-120043

Azevedo, J., Garcia, D., Pontier, D., Ohnesorge, S., Yu, A., Garcia, S., Braun, L., Bergdoll, M., Hakimi, M.A., Lagrange, T., Voinnet, O., 2010. Argonaute quenching and global changes in Dicer homeostasis caused by a pathogen-encoded GW repeat protein. Genes Dev. 24, 904–915. https://doi.org/10.1101/gad.1908710

Bakker, E.G., Toomajian, C., Kreitman, M., Bergelson, J., n.d. A Genome-Wide Survey of R Gene Polymorphisms in Arabidopsis W. https://doi.org/10.1105/tpc.106.042614

Bally, J., Nakasugi, K., Jia, F., Jung, H., Ho, S.Y.W., Wong, M., Paul, C.M., Naim, F., Wood, C.C., Crowhurst, R.N., Hellens, R.P., Dale, J.L., Waterhouse, P.M., 2015. The extremophile Nicotiana benthamiana has traded viral defence for early vigour. Nat. Plants 1, 1–6. https://doi.org/10.1038/nplants.2015.165

Balogun, O.S., 2003. Patterns of disease manifestation in tomato seedlings singly or doubly infected with Potato X potexvirus and Tobacco Mosaic tobamovirus.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using **lme4**. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Baumberger, N., Baulcombe, D.C., 2005. Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. Proc. Natl. Acad. Sci. U. S. A. 102, 11928–33.

Bayne, E.H., Rakitina, D. V., Morozov, S.Y., Baulcombe, D.C., 2005. Cell-to-cell movement of Potato Potexvirus X is dependent on suppression of RNA silencing. Plant J. 44, 471–482. https://doi.org/10.1111/j.1365-313X.2005.02539.x

Bejerman, N., Mann, K.S., Dietzgen, R.G., 2016. Alfalfa dwarf cytorhabdovirus P protein is a local and systemic RNA silencing supressor which inhibits programmed RISC activity and prevents transitive amplification of RNA silencing. Virus Res. 224, 19–28.

https://doi.org/10.1016/j.virusres.2016.08.008

Belgorodski, N.K.S.K.T., 2017. Package “rriskDistributions” Type Package Title Fitting Distributions to Given Data or Known Quantiles.

Bellaoui, M., Keddie, J.S., Gruissem, W., 2003. DCL is a plant-specific protein required for plastid ribosomal RNA processing and embryo development. Plant Mol. Biol. 53, 531–543.

Bendahmane, A., Kanyuka, K., Baulcombe, D.C., 1999. The Rx Gene from Potato Controls Separate Virus Resistance and Cell Death Responses. Plant Cell 11, 781–791.

Bendahmane, A., Querci, M., Kanyuka, K., Baulcombe, D.C., 2000. Agrobacterium transient expression system as a tool for the isolation of disease resistance genes: application to the Rx2 locus in potato. Plant J. 21, 73–81. https://doi.org/10.1046/j.1365-313x.2000.00654.x

Bengyella, L., Waikhom, S.D., Allie, F., Rey, C., 2015. Virus tolerance and recovery from viral induced-symptoms in plants are associated with transcriptome reprograming. Plant Mol. Biol. 89, 243–252. https://doi.org/10.1007/s11103-015-0362-6

Best, A., White, A., Boots, M., 2008. Maintenance of host variation in tolerance to pathogens and parasites. Proc. Natl. Acad. Sci. U. S. A. 105, 20786–91. https://doi.org/10.1073/pnas.0809558105

Bhattacharjee, S., Zamora, A., Azhar, M.T., Sacco, M.A., Lambert, L.H., Moffett, P., 2009. Virus resistance induced by NB-LRR proteins involves Argonaute4-dependent translational control. Plant J. 58, 940–951. https://doi.org/10.1111/j.1365-313X.2009.03832.x

Blevins, T., Rajeswaran, R., Shivaprasad, P. V., Beknazariants, D., Si-Ammour, A., Park, H.S., Vazquez, F., Robertson, D., Meins, F., Hohn, T., Pooggin, M.M., 2006. Four plant Dicers mediate viral small RNA biogenesis and DNA virus induced silencing. Nucleic Acids Res. 34, 6233–6246.

Bohmert, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M., Benning, C., 1998. AGO1 defines a novel locus of Arabidopsis controlling leaf development. EMBO J. 17, 170–180.

Bologna, N.G., Voinnet, O., 2014. The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis. Annu. Rev. Plant Biol. 65.

https://doi.org/10.1146/annurev-arplant-050213-035728

Borevitz, J.O., Hazen, S.P., Michael, T.P., Morris, G.P., Baxter, I.R., Hu, T.T., Chen, H., Werner, J.D., Nordborg, M., Salt, D.E., Kay, S.A., Chory, J., Weigel, D., Jones, J.D.G., Ecker, J.R., 2007. Genome-wide patterns of single-feature polymorphism in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 104, 12057–62. https://doi.org/10.1073/pnas.0705323104

Borges, F., Martienssen, R.A., 2015. The expanding world of small RNAs in plants. Nat. Publ. Gr. 16. https://doi.org/10.1038/nrm4085

Bortolamiol, D., Pazhouhandeh, M., Marrocco, K., Genschik, P., Ronique Ziegler-Graff, V., 2007. Report The Polerovirus F Box Protein P0 Targets ARGONAUTE1 to Suppress RNA Silencing. Curr. Biol. 17, 1615–1621. https://doi.org/10.1016/j.cub.2007.07.061

Boualem, A., Dogimont, C., Bendahmane, A., 2016. The battle for survival between viruses and their host plants. Curr. Opin. Virol. https://doi.org/10.1016/j.coviro.2015.12.001

Bouché, N., Lauressergues, D., Gasciolli, V., Vaucheret, H., 2006. An antagonistic function for Arabidopsis DCL2 in development and a new function for DCL4 in generating viral siRNAs. EMBO J. 25, 3347–3356. https://doi.org/10.1038/sj.emboj.7601217

Boutrot, F., Zipfel, C., 2017. Function, Discovery, and Exploitation of Plant Pattern Recognition Receptors for Broad-Spectrum Disease Resistance. Annu. Rev. Phytopathol. 55, 257–286. https://doi.org/10.1146/annurev-phyto-080614-120106

Boyes, D.C., Zayed, A.M., Ascenzi, R., Mccaskill, A.J., Hoffman, N.E., Davis, K.R., Görlach, J., 2001. Growth Stage-Based Phenotypic Analysis of Arabidopsis: A Model for High Throughput Functional Genomics in Plants, The Plant Cell.

Brodersen, P., Sakvarelidze-Achard, L., Bruun-Rasmussen, M., Dunoyer, P., Yamamoto, Y.Y., Sieburth, L., Voinnet, O., 2008. Widespread Translational Inhibition by Plant miRNAs and siRNAs. Science (80-.). 320, 1185–1190. https://doi.org/10.1126/science.1159151

Brosseau, C., Adurogbangba, A., Roussin-Léveillée, C., Zhao, Z., Biga, S., Moffett, P., 2019. Natural variation in the Arabidopsis AGO2 gene is associated with susceptibility to potato virus X. bioRxiv 746628. https://doi.org/10.1101/746628

Brosseau, C., Moffett, P., 2015. Functional and Genetic Analysis Identify a Role for Arabidopsis ARGONAUTE5 in Antiviral RNA Silencing. Plant Cell 27, 1742–54. https://doi.org/10.1105/tpc.15.00264

Brosseau, C., Oirdi, M. El, Adurogbangba, A., Ma, X., Moffett, P., 2016. Antiviral Defense Involves AGO4 in an Arabidopsis–Potexvirus Interaction. MPMI 29, 878–888. https://doi.org/10.1094/MPMI-09-16-0188-R

Bucher, E., Lohuis, D., van Poppel, P.M.J.A., Geerts-Dimitriadou, C., Goldbach, R., Prins, M., 2006. Multiple virus resistance at a high frequency using a single transgene construct. J. Gen. Virol. 87, 3697–3701. https://doi.org/10.1099/vir.0.82276-0

Buchmann, R.C., Asad, S., Wolf, J.N., Mohannath, G., Bisaro, D.M., 2009. Geminivirus AL2 and L2 Proteins Suppress Transcriptional Gene Silencing and Cause Genome-Wide Reductions in Cytosine Methylation □. J. Virol. 83, 5005–5013. https://doi.org/10.1128/JVI.01771-08

Cao, J., Schneeberger, K., Ossowski, S., Günther, T., Bender, S., Fitz, J., Koenig, D., Lanz, C., Stegle, O., Lippert, C., Wang, X., Ott, F., Müller, J., Alonso-Blanco, C., Borgwardt, K., Schmid, K.J., Weigel, D., 2011. Whole-genome sequencing of multiple Arabidopsis thaliana populations. Nat. Genet. 43, 956–965. https://doi.org/10.1038/ng.911

Carbonell, A., Carrington, J.C., 2015. Antiviral roles of plant ARGONAUTES. Curr. Opin. Plant Biol. 27, 111–117. https://doi.org/10.1016/j.pbi.2015.06.013

Carbonell, A., Fahlgren, N., Garcia-Ruiz, H., Gilbert, K.B., Montgomery, T.A., Nguyen, T., Cuperus, J.T., Carrington, J.C., 2012. Functional analysis of three Arabidopsis ARGONAUTES using slicer-defective mutants. Plant Cell 24, 3613–29. https://doi.org/10.1105/tpc.112.099945

Carthew, R.W., Sontheimer, E.J., 2009. Origins and Mechanisms of miRNAs and siRNAs. Cell 136, 642–55. https://doi.org/10.1016/j.cell.2009.01.035

Chae, E., Tran, D.T.N., Weigel, D., 2016. Cooperation and Conflict in the Plant Immune System. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1005452

Chellappan, P., Vanitharani, R., Ogbe, F., Fauquet, C.M., 2005. Effect of temperature on

geminivirus-induced RNA silencing in plants. Plant Physiol. 138, 1828–41. https://doi.org/10.1104/pp.105.066563

Chen, H., Zhang, L., Yu, K., Wang, A., 2015. Pathogenesis of Soybean mosaic virus in soybean carrying Rsv1 gene is associated with miRNA and siRNA pathways, and breakdown of AGO1 homeostasis. Virology 476, 395–404. https://doi.org/10.1016/j.virol.2014.12.034

Cheng, X., Wang, A., 2017. The Potyvirus Silencing Suppressor Protein VPg Mediates Degradation of SGS3 via Ubiquitination and Autophagy Pathways. J. Virol. 91. https://doi.org/10.1128/jvi.01478-16

Chisholm, S.T., Coaker, G., Day, B., Staskawicz, B.J., 2006. Host-microbe interactions: Shaping the evolution of the plant immune response. Cell.

Chiu, M.-H., Chen, I.-H., Baulcombe, D.C., Tsai, C.-H., 2010. The silencing suppressor P25 of Potato virus X interacts with Argonaute1 and mediates its degradation through the proteasome pathway. Mol. Plant Pathol. 11, 641–9. https://doi.org/10.1111/j.1364-3703.2010.00634.x

Cho, Y.B., Jones, S.I., Vodkin, L.O., 2017. Mutations in Argonaute5 Illuminate Epistatic Interactions of the K1 and I Loci Leading to Saddle Seed Color Patterns in Glycine max. Plant Cell 29, tpc.00162.2017. https://doi.org/10.1105/tpc.17.00162

Clark, R.M., Schweikert, G., Toomajian, C., Ossowski, S., Zeller, G., Shinn, P., Warthmann, N., Hu, T.T., Fu, G., Hinds, D.A., Chen, H., Frazer, K.A., Huson, D.H., Scholkopf, B., Nordborg, M., Ratsch, G., Ecker, J.R., Weigel, D., 2007. Common Sequence Polymorphisms Shaping Genetic Diversity in Arabidopsis thaliana. Science (80-.). 317, 338–342. https://doi.org/10.1126/science.1138632

Cook, D.E., Mesarich, C.H., Thomma, B.P.H.J., 2015. Understanding Plant Immunity as a Surveillance System to Detect Invasion. Annu. Rev. Phytopathol 53, 541–63. https://doi.org/10.1146/annurev-phyto-080614-120114

Cooley, M.B., Pathirana, S., Wu, H.J., Kachroo, P., Klessig, D.F., 2000. Members of the Arabidopsis HRT/RPP8 family of resistance genes confer resistance to both viral and oomycete pathogens. Plant Cell 12, 663–76. https://doi.org/10.1105/tpc.12.5.663

Cosson, P., Schurdi-Levraud, V., Le, Q.H., Sicard, O., Caballero, M., Roux, F., Le Gall, O., Candresse, T., Revers, F., 2012. The RTM resistance to potyviruses in Arabidopsis thaliana: natural variation of the RTM genes and evidence for the implication of additional genes. PLoS One 7, e39169. https://doi.org/10.1371/journal.pone.0039169

Csorba, T., Kontra, L., Burgyán, J., 2015. Viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. Virology. https://doi.org/10.1016/j.virol.2015.02.028

Csorba, T., Lózsa, R., Hutvágner, G., Burgyán, J., 2010. Polerovirus protein P0 prevents the assembly of small RNA-containing RISC complexes and leads to degradation of ARGONAUTE1. Plant J. 62, 463–472. https://doi.org/10.1111/j.1365-313X.2010.04163.x

Curtin, S.J., Michno, J.-M., Campbell, B.W., Gil-Humanes, J., Mathioni, S.M., Hammond, R., Gutierrez-Gonzalez, J.J., Donohue, R.C., Kantar, M.B., Eamens, A.L., Meyers, B.C., Voytas, D.F., Stupar, R.M., 2016. MicroRNA Maturation and MicroRNA Target Gene Expression Regulation Are Severely Disrupted in Soybean dicer-like1 Double Mutants. Genes, Genomes, Genet. 6, 423–433.

Dempsey, D.A., Klessig, D.F., 2012. SOS - too many signals for systemic acquired resistance? Trends Plant Sci.

Dennis Gonsalves, 2004. Transgenic Papaya in Hawaii and Beyond.

Derrien, B., Baumberger, N., Schepetilnikov, M., Viotti, C., De Cillia, J., Ziegler-Graff, V., Isono, E., Schumacher, K., Genschik, P., 2012. Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. Proc. Natl. Acad. Sci. 109, 15942–15946. https://doi.org/10.1073/pnas.1209487109

Di Serio, F., Schö, H., Iglesias, A., Tarina, C., Bouldoires, E., Meins, F., Phillips, R.L., 2001. Sense-and antisense-mediated gene silencing in tobacco is inhibited by the same viral suppressors and is associated with accumulation of small RNAs. PNAS 98, 6506–6510.

Diao, P., Zhang, Q., Sun, H., Ma, W., Cao, A., Yu, R., Wang, J., Niu, Y., Wuriyanghan, H., Diao, P., Zhang, Q., Sun, H., Ma, W., Cao, A., Yu, R., Wang, J., Niu, Y., Wuriyanghan, H., 2019. miR403a and SA Are Involved in NbAGO2 Mediated Antiviral Defenses Against TMV Infection in Nicotiana benthamiana. Genes (Basel). 10, 526.

https://doi.org/10.3390/genes10070526

Dinesh-Kumar, S.P., Tham, W.-H., Baker, B.J., Gajiwala, P., Pasion, R., Whalen, M.C., Stall, R.E., Staskawicz, B.J., 2000. Structure-function analysis of the tobacco mosaic virus resistance gene N. Proc. Natl. Acad. Sci. 97, 14789–14794. https://doi.org/10.1073/pnas.97.26.14789

Donaire, L., Wang, Y., Gonzalez-Ibeas, D., Mayer, K.F., Aranda, M.A., Llave, C., 2009. Deep-sequencing of plant viral small RNAs reveals effective and widespread targeting of viral genomes. Virology 392, 203–214. https://doi.org/10.1016/j.virol.2009.07.005

Duan, C.-G., Fang, Y.-Y., Zhou, B.-J., Zhao, J.-H., Hou, W.-N., Zhu, H., Ding, S.-W., Guo, H.-S., 2012. Suppression of Arabidopsis ARGONAUTE1-Mediated Slicing, Transgene-Induced RNA Silencing, and DNA Methylation by Distinct Domains of the Cucumber mosaic virus 2b Protein. Plant Cell. https://doi.org/10.1105/tpc.111.092718

Duan, C.-G., Zhang, H., Tang, K., Zhu, X., Qian, W., Hou, Y.-J., Wang, B., Lang, Z., Zhao, Y., Wang, X., Wang, P., Zhou, J., Liang, G., Liu, N., Wang, C., Zhu, J.-K., 2015. Specific but interdependent functions for Arabidopsis AGO4 and AGO6 in RNA-directed DNA methylation. EMBO J. 34, 581–592. https://doi.org/10.15252/embj

Dunoyer, P., Schott, G., Himber, C., Meyer, D., Takeda, A., Carrington, J.C., Voinnet, O., 2010. Small RNA Duplexes Function as Mobile Silencing Signals Between Plant Cells. Science (80-.). 328.

Dzianott, A., Sztuba-Solińska, J., Bujarski, J.J., 2012a. Mutations in the Antiviral RNAi Defense Pathway Modify Brome mosaic virus RNA Recombinant Profiles. Mol. Plant-Microbe Interact. 25, 97–106. https://doi.org/10.1094/MPMI-05-11-0137

Dzianott, A., Sztuba-Solińska, J., Bujarski, J.J., 2012b. Mutations in the Antiviral RNAi Defense Pathway Modify Brome mosaic virus RNA Recombinant Profiles 25, 97–106.

Eamens, A., Wang, M.-B., Smith, N.A., Waterhouse, P.M., 2008. RNA silencing in plants: yesterday, today, and tomorrow. Plant Physiol. 147, 456–68. https://doi.org/10.1104/pp.108.117275

El-Shami, M., Pontier, D., Lahmy, S., Braun, L., Picart, C., Vega, D., Hakimi, M.A., Jacobsen,

S.E., Cooke, R., Lagrange, T., 2007. Reiterated WG/GW motifs form functionally and evolutionarily conserved ARGONAUTE-binding platforms in RNAi-related components. Genes Dev. 21, 2539–2544. https://doi.org/10.1101/gad.451207

El Oirdi, M., El Rahman, T.A., Rigano, L., El Hadrami, A., Rodriguez, M.C., Daayf, F., Vojnov, A., Bouarab, K., 2011. *Botrytis cinerea* Manipulates the Antagonistic Effects between Immune Pathways to Promote Disease Development in Tomato. Plant Cell 23, 2405–2421. https://doi.org/10.1105/tpc.111.083394

Elsharkawy, M.M., Shimizu, M., Takahashi, H., Ozaki, K., Hyakumachi, M., 2013. Induction of Systemic Resistance against Cucumber mosaic virus in Arabidopsis thaliana by Trichoderma asperellum SKT-1. Plant Pathol. J. 29, 193. https://doi.org/10.5423/PPJ.SI.07.2012.01

Enard, D., Cai, L., Gwennap, C., Petrov, D.A., 2016. Viruses are a dominant driver of protein adaptation in mammals. Elife 5, 1–25. https://doi.org/10.7554/eLife.12469

Eun, C., Lorkovic, Z.J., Naumann, U., Long, Q., Havecker, E.R., Simon, S.A., Meyers, B.C., Matzke, A.J.M., Matzke, M., Freitag, M., 2011. AGO6 Functions in RNA-Mediated Transcriptional Gene Silencing in Shoot and Root Meristems in Arabidopsis thaliana. https://doi.org/10.1371/journal.pone.0025730

Fagard, M., Boutet, S., Morel, J.-B., Bellini, C., Vaucheret, H., 2000. AGO1, QDE-2, and RDE-1 are required for post-transcriptional gene silencing in plants , quelling in fungi , and RNA interference in animals. Proc. Natl. Acad. Sci. 97, 11650–11654.

Fang, X., Qi, Y., 2016. RNAi in Plants: An Argonaute-Centered View. Plant Cell 28, 272–85. https://doi.org/10.1105/tpc.15.00920

Fang, Y.-Y., Zhao, J.-H., Liu, S.-W., Wang, S., Duan, C.-G., Guo, H.-S., 2016. CMV2b-AGO Interaction Is Required for the Suppression of RDR-Dependent Antiviral Silencing in Arabidopsis. Front. Microbiol. 7, 1329. https://doi.org/10.3389/fmicb.2016.01329

Fátyol, K., Ludman, M., Burgyán, J., P.D., Z., V., S., T., T., D.J., P., H., V., W.M., K., R., C., S., C., G.J., H., Y., Q., 2016. Functional dissection of a plant Argonaute. Nucleic Acids Res. 44, 1384–1397. https://doi.org/10.1093/nar/gkv1371

Feng, L., Duan, C.-G., Guo, H.-S., 2013. Inhibition of *in vivo* Slicer activity of Argonaute protein 1 by the viral 2b protein independent of its dsRNA-binding function. Mol. Plant Pathol. 14, 617–622. https://doi.org/10.1111/mpp.12033

Ferreira, S.A., Pitz, K.Y., 2002. Virus Coat Protein Transgenic Papaya Provides Practical Control of Papaya ringspot virus in Hawaii.

Frost, K.E., Groves, R.L., Charkowski, A.O., 2013. Integrated Control of Potato Pathogens Through Seed Potato Certification and Provision of Clean Seed Potatoes. https://doi.org/10.1094/PDIS-05-13-0477-FE

Fusaro, A.F., Matthew, L., Smith, N.A., Curtin, S.J., Dedic-Hagan, J., Ellacott, G.A., Watson, J.M., Wang, M.-B., Brosnan, C., Carroll, B.J., Waterhouse, P.M., 2006. RNA interference-inducing hairpin RNAs in plants act through the viral defence pathway. EMBO Rep. 7, 1168–75. https://doi.org/10.1038/sj.embor.7400837

Garcia-Ruiz, H., Carbonell, A., Hoyer, J.S., Fahlgren, N., Gilbert, K.B., Takeda, A., Giampetruzzi, A., Ruiz, M.T.G., Mcginn, M.G., Lowery, N., Martinez Baladejo, M.T., Carrington, J.C., -Ruiz, G., Carbonell, H., Js, H., Ding, B., 2015. Roles and Programming of Arabidopsis ARGONAUTE Proteins during Turnip Mosaic Virus Infection. PLoS Pathog 11. https://doi.org/10.1371/journal.ppat.1004755

Garcia-Ruiz, H., Takeda, A., Chapman, E.J., Sullivan, C.M., Fahlgren, N., Brempelis, K.J., Carrington, J.C., 2010. Arabidopsis RNA-Dependent RNA Polymerases and Dicer-Like Proteins in Antiviral Defense and Small Interfering RNA Biogenesis during Turnip Mosaic Virus Infection. Plant Cell 22, 481–496. https://doi.org/10.1105/tpc.109.073056

Gargouri-Bouzid, R., Jaoua, L., Rouis, S., Saídi, M.N., Bouaziz, D., Ellouz, R., 2006. PVY-Resistant Transgenic Potato Plants Expressing an Anti-NIa Protein scFv Antibody. Mol. Biotechnol. 33, 133–140. https://doi.org/10.1385/MB:33:2:133

Ghoshal, B., Sanfaçon, H., 2014. Temperature-dependent symptom recovery in Nicotiana benthamiana plants infected with tomato ringspot virus is associated with reduced translation of viral RNA2 and requires ARGONAUTE 1. Virology 456–457, 188–197. https://doi.org/10.1016/J.VIROL.2014.03.026

Gill, U.S., Lee, S., Mysore, K.S., 2015. Host Versus Nonhost Resistance: Distinct Wars with Similar Arsenals. Phytopathology. https://doi.org/10.1094/phyto-11-14-0298-rvw

Giner, A., Lakatos, L., García-Chapa, M., López-Moya, J.J., Burgyán, J., 2010. Viral Protein Inhibits RISC Activity by Argonaute Binding through Conserved WG/GW Motifs. PLoS Pathog. 6, e1000996. https://doi.org/10.1371/journal.ppat.1000996

Gonzalez-Gaitan, M., Jessell, T., Johnston, L., Laufer, E., McCabe, B., Struhl, G., Deleris, A., Gallego-Bartolome, J., Bao, J., Kasschau, K.D., Carrington, J.C., Voinnet, O., 2004. Hierarchical Action and Inhibition of Plant Dicer-Like Proteins in Antiviral Defense. Science (80-.). 305, 1462–322. https://doi.org/10.1126/science.1128650

González, I., Martínez, L., Rakitina, D. V., Lewsey, M.G., Atencio, F.A., Llave, C., Kalinina, N.O., Carr, J.P., Palukaitis, P., Canto, T., 2010. Cucumber mosaic virus 2b protein subcellular targets and interactions: Their significance to RNA silencing suppressor activity. Mol. Plant-Microbe Interact. 23, 294–303. https://doi.org/10.1094/MPMI-23-3-0294

González, I., Rakitina, D., Semashko, M., Taliansky, M., Praveen, S., Palukaitis, P., Carr, J.P., Kalinina, N., Canto, T., 2012. RNA binding is more critical to the suppression of silencing function of Cucumber mosaic virus 2b protein than nuclear localization. RNA 18, 771–782. https://doi.org/10.1261/rna.031260.111

Goto, K., Kobori, T., Kosaka, Y., Natsuaki, T., Masuta, C., 2007. Characterization of Silencing Suppressor 2b of Cucumber Mosaic Virus Based on Examination of its Small RNA-Binding Abilities. Plant Cell Physiol. 48, 1050–1060. https://doi.org/10.1093/pcp/pcm074

Gross Juergen, L.U., 2015. Tests for Normality [R package nortest version 1.0-4].

Ha, M., Kim, V.N., 2014. Regulation of microRNA biogenesis. Nat. Rev. Mol. Cell Biol. 15, 509–524.

Haas, G., Azevedo, J., Moissiard, G., Le Geldreich, A., Himber, C., Bureau, M., Fukuhara, T., Keller, M., Voinnet, O., 2008. Nuclear import of CaMV P6 is required for infection and suppression of the RNA silencing factor DRB4. EMBO J. 27129, 2102–2112.

Hamera, S., Song, X., Su, L., Chen, X., Fang, R., 2012. Cucumber mosaic virus suppressor 2b binds to AGO4-related small RNAs and impairs AGO4 activities. Plant J. 69, 104–115.

Harvey, J.J.W., Lewsey, M.G., Patel, K., Westwood, J., Heimstädt, S., Carr, J.P., Baulcombe, D.C., 2011. An antiviral defense role of AGO2 in plants. PLoS One 6.

Hashimoto, M., Neriya, Y., Keima, T., Iwabuchi, N., Koinuma, H., Hagiwara-Komoda, Y., Ishikawa, K., Himeno, M., Maejima, K., Yamaji, Y., Namba, S., 2016. EXA1, a GYF domain protein, is responsible for loss-of-susceptibility to plantago asiatica mosaic virus in *Arabidopsis thaliana*. Plant J. 88, 120–131. https://doi.org/10.1111/tpj.13265

Hauptmann, J., Schraivogel, D., Bruckmann, A., Manickavel, S., Jakob, L., Eichner, N., Pfaff, J., Urban, M., Sprunck, S., Hafner, M., Tuschl, T., Deutzmann, R., Meister, G., 2015. Biochemical isolation of Argonaute protein complexes by Ago-APP. Proc. Natl. Acad. Sci. U. S. A. 112, 11841–5. https://doi.org/10.1073/pnas.1506116112

Havecker, E.R., Wallbridge, L.M., Hardcastle, T.J., Bush, M.S., Kelly, K.A., Dunn, R.M., Schwach, F., Doonan, J.H., Baulcombe, D.C., 2010. The Arabidopsis RNA-directed DNA methylation argonautes functionally diverge based on their expression and interaction with target loci. Plant Cell 22, 321–34. https://doi.org/10.1105/tpc.109.072199

Hernández-Lagana, E., Rodríguez-Leal, D., Lúa, J., Vielle-Calzada, J.P., 2016. A multigenic network of ARGONAUTE4 clade members controls early megaspore formation in arabidopsis. Genetics 204, 1045–1056. https://doi.org/10.1534/genetics.116.188151

Hily, J.M., García, A., Moreno, A., Plaza, M., Wilkinson, M.D., Fereres, A., Fraile, A., García-Arenal, F., 2014. The Relationship between Host Lifespan and Pathogen Reservoir Potential: An Analysis in the System Arabidopsis thaliana-Cucumber mosaic virus. PLoS Pathog. 10, e1004492. https://doi.org/10.1371/journal.ppat.1004492

Hohn, T., Vazquez, F., 2011. RNA silencing pathways of plants: Silencing and its suppression by plant DNA viruses ☆. BBA - Gene Regul. Mech. 1809, 588–600. https://doi.org/10.1016/j.bbagrm.2011.06.002

Howell, M.D., Fahlgren, N., Chapman, E.J., Cumbie, J.S., Sullivan, C.M., Givan, S.A., Kasschau, K.D., Carrington, J.C., 2007. Genome-wide analysis of the RNA-DEPENDENT RNA POLYMERASE6/DICER-LIKE4 pathway in Arabidopsis reveals dependency on miRNA- and tasiRNA-directed targeting. Plant Cell 19, 926–42.

https://doi.org/10.1105/tpc.107.050062

Hu, P., Zhao, H., Zhu, P., Xiao, Y., Miao, W., Wang, Y., Jin, H., 2019. Dual regulation of Arabidopsis AGO2 by arginine methylation. Nat. Commun. 10, 844. https://doi.org/10.1038/s41467-019-08787-w

Hunter, C., 2006. Trans-acting siRNA-mediated repression of ETTIN and ARF4 regulates heteroblasty in Arabidopsis. Development 133, 2973–2981. https://doi.org/10.1242/dev.02491

Inoue-Nagata, A.K., Lima, M.F., Gilbertson, R.L., Inoue-Nagata, A.K., Lima, M.F., Gilbertson, R.L., 2016. A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. Hortic. Bras. 34, 8–18. https://doi.org/10.1590/S0102-053620160000100002

Ivanov, K.I., Eskelin, K., Bašić, M., De, S., Lõhmus, A., Varjosalo, M., Mäkinen, K., 2016a. Molecular insights into the function of the viral RNA silencing suppressor HCPro. Plant J. 85, 30–45. https://doi.org/10.1111/tpj.13088

Ivanov, K.I., Eskelin, K., Bašić, M., De, S., Lõhmus, A., Varjosalo, M., Mäkinen, K., 2016b. Molecular insights into the function of the viral RNA silencing suppressor HCPro. Plant J. 85, 30–45. https://doi.org/10.1111/tpj.13088

Ivanov, K.I., Eskelin, K., Lõ, A., Mä Kinen Correspondence, K., Mä, K., 2014. Molecular and cellular mechanisms underlying potyvirus infection. J. Gen. Virol. 95, 1415–1429. https://doi.org/10.1099/vir.0.064220-0

Iwasaki, S., Kobayashi, M., Yoda, M., Sakaguchi, Y., Katsuma, S., Suzuki, T., Tomari, Y., 2010. Hsc70/Hsp90 Chaperone Machinery Mediates ATP-Dependent RISC Loading of Small RNA Duplexes. Mol. Cell 39, 292–299. https://doi.org/10.1016/j.molcel.2010.05.015

Jacquemond, M., 2012. Cucumber Mosaic Virus. Adv. Virus Res. 84, 439–504. https://doi.org/10.1016/B978-0-12-394314-9.00013-0

Jain, R.K., Pappu, H.R., Pappu, S.S., Varma, A., Ram, R.D., 1998. Molecular characterisation of papaya ringspot potyvirus isolates from India. Ann. Appl. Biol. 132, 413–425. https://doi.org/10.1111/j.1744-7348.1998.tb05218.x

Jamous, R.M., Boonrod, K., Fuellgrabe, M.W., Ali-Shtayeh, M.S., Krczal, G., Wassenegger, M., 2011. The helper component-proteinase of the Zucchini yellow mosaic virus inhibits the Hua Enhancer 1 methyltransferase activity in vitro. J. Gen. Virol. 92, 2222–2226.

Jaubert, M., Bhattacharjee, S., Mello, A.F.S., Perry, K.L., Moffett, P., 2011. ARGONAUTE2 Mediates RNA-Silencing Antiviral Defenses against Potato virus X in Arabidopsis 1[W][OA]. Plant Physiol. 156. https://doi.org/10.1104/pp.111.178012

Jenner, C.E., Wang, X., Ponz, F., Walsh, J.A., 2002. A fitness cost for Turnip mosaic virus to overcome host resistance. Virus Res. 86, 1–6. https://doi.org/10.1016/S0168-1702(02)00031-X

Jens Tilsner, O.L., Louveaux, M., Ian M. Roberts, Sean N. Chapman, and Karl J. Oparka, 2013. Replication and trafficking of a plant virus are coupled at the entrances of plasmodesmata. J. Cell Biol 201, 981–995. https://doi.org/10.1083/jcb.201304003

Jones, J.D.G., Dangl, J.L., 2006. The plant immune system. Nature 444, 323–329.

Jones, J.D.G., Vance, R.E., Dangl, J.L., 2016. Intracellular innate immune surveillance devices in plants and animals. Science (80-.). 354, 1117. https://doi.org/10.1126/science.aaf6395

Jouannet, V., Moreno, A.B., Elmayan, T., Vaucheret, H., Crespi, M.D., Maizel, A., 2012. Cytoplasmic Arabidopsis AGO7 accumulates in membrane-associated siRNA bodies and is required for ta-siRNA biogenesis. EMBO J. 31, 1704–1713. https://doi.org/10.1038/emboj.2012.20

Kapoor, M., Arora, R., Lama, T., Nijhawan, A., Khurana, J.P., Tyagi, A.K., Kapoor, S., 2008. Genome-wide identification, organization and phylogenetic analysis of Dicer-like, Argonaute and RNA-dependent RNA Polymerase gene families and their expression analysis during reproductive development and stress in rice. BMC Genomics 9. https://doi.org/10.1186/1471-2164-9-451

Kasschau, K.D., Carrington, J.C., 1998. A counterdefensive strategy of plant viruses: Suppression of posttranscriptional gene silencing. Cell 95, 461–470. https://doi.org/10.1016/S0092-8674(00)81614-1

Keima, T., Hagiwara-Komoda, Y., Hashimoto, M., Neriya, Y., Koinuma, H., Iwabuchi, N.,

Nishida, S., Yamaji, Y., Namba, S., 2017. Deficiency of the eIF4E isoform nCBP limits the cell-to-cell movement of a plant virus encoding triple-gene-block proteins in Arabidopsis thaliana. Sci. Rep. 7, 1–13. https://doi.org/10.1038/srep39678

Kidner, C.A., Martienssen, R.A., 2005. The role of ARGONAUTE1 (AGO1) in meristem formation and identity. Dev. Biol. 280, 504–517. https://doi.org/10.1016/J.YDBIO.2005.01.031

Kim, V.N., 2005. Small RNAs: classification, biogenesis, and function. Mol. Cells 19, 1–15. https://doi.org/806 [pii]

Kirino, Y., Kim, N., de Planell-Saguer, M., Khandros, E., Chiorean, S., Klein, P.S., Rigoutsos, I., Jongens, T.A., Mourelatos, Z., 2009. Arginine methylation of Piwi proteins catalysed by dPRMT5 is required for Ago3 and Aub stability. Nat. Cell Biol. 11, 652–658. https://doi.org/10.1038/ncb1872

Kirino, Y., Vourekas, A., Sayed, N., de Lima Alves, F., Thomson, T., Lasko, P., Rappsilber, J., Jongens, T.A., Mourelatos, Z., 2010. Arginine methylation of Aubergine mediates Tudor binding and germ plasm localization. Rna 16, 70–78. https://doi.org/10.1261/rna.1869710

Koenig, D., Jimenez-Gomez, J.M., Kimura, S., Fulop, D., Chitwood, D.H., Headland, L.R., Kumar, R., Covington, M.F., Devisetty, U.K., Tat, A. V., Tohge, T., Bolger, A., Schneeberger, K., Ossowski, S., Lanz, C., Xiong, G., Taylor-Teeples, M., Brady, S.M., Pauly, M., Weigel, D., Usadel, B., Fernie, A.R., Peng, J., Sinha, N.R., Maloof, J.N., 2013. Comparative transcriptomics reveals patterns of selection in domesticated and wild tomato, Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.1309606110

Kontra, L., Csorba, T., Tavazza, M., Lucioli, A., Tavazza, R., Moxon, S., Tisza, V., Medzihradszky, A., Turina, M., Burgyán, J., 2016. Distinct Effects of p19 RNA Silencing Suppressor on Small RNA Mediated Pathways in Plants. PLoS Pathog. 12, e1005935. https://doi.org/10.1371/journal.ppat.1005935

Koornneef, M., Alonso-Blanco, C., Vreugdenhil, D., 2004. NATURALLY OCCURRING GENETIC VARIATION IN *ARABIDOPSIS THALIANA*. Annu. Rev. Plant Biol. 55, 141–

172. https://doi.org/10.1146/annurev.arplant.55.031903.141605

Kosakovsky Pond, S.L., Frost, S.D.W., 2005. Not So Different After All: A Comparison of Methods for Detecting Amino Acid Sites Under Selection. Mol. Biol. Evol. 22, 1208–1222. https://doi.org/10.1093/molbev/msi105

Kurihara, Y., Watanabe, Y., 2004. Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. PNAS 101, 12753–12758.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685. https://doi.org/10.1038/227680a0

Lakatos, L., Szittya, G., Silhavy, D., Burgyán, J., 2004. Molecular mechanism of RNA silencing suppression mediated by p19 protein of tombusviruses. EMBO J. 23, 876–884.

Lario, L.D., Ramirez-Parra, E., Gutierrez, C., Spampinato, C.P., Casati, P., 2013. ANTI-SILENCING FUNCTION1 Proteins Are Involved in Ultraviolet-Induced DNA Damage Repair and Are Cell Cycle Regulated by E2F Transcription Factors in Arabidopsis 1[C][W][OA]. Plant Physiol. 162, 1164–1177. https://doi.org/10.1104/pp.112.212837

Lau, W., Fischbach, M.A., Osbourn, A., Sattely, E.S., 2014. Key applications of plant metabolic engineering. PLoS Biol. 12, e1001879. https://doi.org/10.1371/journal.pbio.1001879

Laufs, J., Trautt, W., Heyraudt, F., Matzeitt, V., Rogersf, S.G., Schellt, J., Gronenborn, B., 1995. In vitro cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. Biochemistry 92, 3879–3883.

Laura R. Newburn, K.A.W., 2015. Cis-acting RNA elements in positive-strand RNA plant virus genomes. Virology 479, 434–443.

Lee, S.-C., Wu, C.-H., Wang, C.-W., 2010. Traffic of a Viral Movement Protein Complex to the Highly Curved Tubules of the Cortical Endoplasmic Reticulum. Traffic 11, 912–930. https://doi.org/10.1111/j.1600-0854.2010.01064.x

Lee, S., Rojas, C.M., Ishiga, Y., Pandey, S., Mysore, K.S., 2013. Arabidopsis heterotrimeric G-proteins play a critical role in host and nonhost resistance against Pseudomonas syringae pathogens. PLoS One 8, e82445. https://doi.org/10.1371/journal.pone.0082445

Lee, S., Whitaker, V.M., Hutton, S.F., 2016. Mini Review: Potential Applications of Non-host Resistance for Crop Improvement. Front. Plant Sci. https://doi.org/10.3389/fpls.2016.00997

Li, C.F., Henderson, I.R., Song, L., Fedoroff, N., Lagrange, T., Jacobsen, S.E., 2008. Dynamic regulation of ARGONAUTE4 within multiple nuclear bodies in Arabidopsis thaliana. PLoS Genet. 4. https://doi.org/10.1371/journal.pgen.0040027

Li, C.F., Pontes, O., El-Shami, M., Henderson, I.R., Bernatavichute, Y. V., Chan, S.W.L., Lagrange, T., Pikaard, C.S., Jacobsen, S.E., 2006. An ARGONAUTE4-Containing Nuclear Processing Center Colocalized with Cajal Bodies in Arabidopsis thaliana. Cell 126, 93–106. https://doi.org/10.1016/j.cell.2006.05.032

Li, F., Wang, A., 2019. RNA-Targeted Antiviral Immunity: More Than Just RNA Silencing. Trends Microbiol. xx, 1–14. https://doi.org/10.1016/j.tim.2019.05.007

Li, F., Zhao, N., Li, Z., Xu, X., Wang, Y., Yang, X., Liu, S.-S., Wang, A., Zhou, X., 2017. A calmodulin-like protein suppresses RNA silencing and promotes geminivirus infection by degrading SGS3 via the autophagy pathway in Nicotiana benthamiana. PLoS Pathog. 13, e1006213. https://doi.org/10.1371/journal.ppat.1006213

Liang Wu, Huan Chen, C.C.& Z.Q.F., 2014. Go in for the kill: How plants deploy effectortriggered immunity to combat pathogens. Virulence 5, 710–721. https://doi.org/10.4161/viru.29755

Liao, Y.-C., Pingli, H., Senzhao, C., Yao, M.-J., Zhang, J.-B., Liu, J.-L., Lia0, Y.-C., 2006. Biotechnology and Genetic Engineering Reviews Plantibodies: A Novel Strategy to Create Pathogen-Resistant Plants 13 Plantibodies: A Novel Strategy to Create Pathogen-Resistant Plants. Biotechnol Genet Eng Rev. 23, 253–71. https://doi.org/10.1080/02648725.2006.10648087

Lico, C., Benvenuto, E., Baschieri, S., 2015. The Two-Faced Potato Virus X: From Plant Pathogen to Smart Nanoparticle. Front. Plant Sci. 6, 1009. https://doi.org/10.3389/fpls.2015.01009

Liu, J., Carmell, M. a, Rivas, F. V, Marsden, C.G., Thomson, J.M., Song, J.-J., Hammond, S.M., Joshua-Tor, L., Hannon, G.J., 2004. Argonaute2 is the catalytic engine of mammalian

RNAi. Science 305, 1437–1441. https://doi.org/10.1126/science.1102513

Liu, Q., Feng, Y., Zhu, Z., 2009. Dicer-like (DCL) proteins in plants. Funct. Integr. Genomics.

Lough, T.J., Lee, R.H., Emerson, S.J., Forster, R.L.S., Lucas, W.J., 2006. Functional analysis of the 5′ untranslated region of potexvirus RNA reveals a role in viral replication and cell-to-cell movement. Virology 351, 455–465. https://doi.org/10.1016/J.VIROL.2006.03.043

Lu, R., Malcuit, T., Moffett, P., Ruiz, M.T., Peart, J., Wu, A.J., Rathjen, J.P., Bendahmane, A., Day, L., Baulcombe, D.C., 2003. High throughput virus-induced gene silencing implicates heat shock protein 90 in plant disease resistance. Embo J 22. https://doi.org/10.1093/emboj/cdg546

Lucht, J.M., 2015. Public Acceptance of Plant Biotechnology and GM Crops. Viruses 7, 4254–81. https://doi.org/10.3390/v7082819

Ludman, M., Burgyán, J., Fátyol, K., 2017a. Crispr/Cas9 Mediated Inactivation of Argonaute 2 Reveals its Differential Involvement in Antiviral Responses. Sci. Rep. 7, 1010. https://doi.org/10.1038/s41598-017-01050-6

Ludman, M., Burgyán, J., Fátyol, K., 2017b. Crispr/Cas9 Mediated Inactivation of Argonaute 2 Reveals its Differential Involvement in Antiviral Responses /631/337/384/2053 /631/449/2169/597 /42/89 /42/44 /38/22 article. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-01050-6

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P., Barton, M.K., 1999. The PINHEAD / ZWILLE gene acts pleiotropically in Arabidopsis development and has overlapping functions with the ARGONAUTE1 gene. Development 481, 469–481.

Ma, J.-B., Ye, K., Patel, D.J., 2004. Structural basis for overhang-specific small interfering RNA recognition by the PAZ domain. Nature 429, 318–22. https://doi.org/10.1038/nature02519

Ma, X., Nicole, M.-C., Meteignier, L.-V., Hong, N., Wang, G., Moffett, P., 2015. Different roles for RNA silencing and RNA processing components in virus recovery and virus-induced gene silencing in plants. J. Exp. Bot. 66, 919–932. https://doi.org/10.1093/jxb/eru447

Mallory, A., Vaucheret, H., 2010. Form, function, and regulation of ARGONAUTE proteins.

Plant Cell 22, 3879–89. https://doi.org/10.1105/tpc.110.080671

Mallory, A.C., Hinze, A., Tucker, M.R., Bouche, N., Gasciolli, V., Elmayan, T., Lauressergues, D., Jauvion, V., Vaucheret, H., Laux, T., 2009. Redundant and Specific Roles of the ARGONAUTE Proteins AGO1 and ZLL in Development and Small RNA- Directed Gene Silencing. PLoS Genet. 5, 1–12. https://doi.org/10.1371/journal.pgen.1000646

Manavella, P.A., Koenig, D., Weigel, D., 2012. Plant secondary siRNA production determined by microRNA-duplex structure. Proc. Natl. Acad. Sci. U. S. A. 109, 2461–6.

Manzano-Piedras, E., Marcer, A., Alonso-Blanco, C., Picó, F.X., 2014. Deciphering the Adjustment between Environment and Life History in Annuals: Lessons from a Geographically-Explicit Approach in Arabidopsis thaliana. PLoS One 9, e87836. https://doi.org/10.1371/journal.pone.0087836

Matić, S., Noris, E., Contin, R., Marian, D., Thompson, J.R., 2019. Engineering partial resistance to cucumber mosaic virus in tobacco using intrabodies specific for the viral polymerase. Phytochemistry 162, 99–108. https://doi.org/10.1016/J.PHYTOCHEM.2019.03.006

Matzke, M. a, Kanno, T., Matzke, A.J.M., 2014. RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants. Annu. Rev. Plant Biol. 1–25. https://doi.org/10.1146/annurev-arplant-043014-114633

Matzke, M.A., Kanno, T., Matzke, A.J.M., 2015. RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants. Annu. Rev. Plant Biol. 66, 243–267. https://doi.org/10.1146/annurev-arplant-043014-114633

Maunoury, N., Vaucheret, H., 2011. AGO1 and AGO2 Act Redundantly in miR408-Mediated Plantacyanin Regulation. PLoS One 6, e28729. https://doi.org/10.1371/journal.pone.0028729

Mauricio, R., 1998. Costs of Resistance to Natural Enemies in Field Populations of the Annual Plant Arabidopsis thaliana Costs of Resistance to Natural Enemies in Field Populations of the Annual Plant Arabidopsis thaliana count for the common observation that, although plant. Source Am. Nat. 151, 20–28. https://doi.org/10.1086/286099

McCue, A.D., Panda, K., Nuthikattu, S., Choudury, S.G., Thomas, E.N., Slotkin, R.K., 2015.

ARGONAUTE 6 bridges transposable element mRNA-derived siRNAs to the establishment of DNA methylation. EMBO J. 34, 20–35. https://doi.org/10.15252/embj.201489499

McHale, L., Tan, X., Koehl, P., Michelmore, R.W., 2006. Plant NBS-LRR proteins: adaptable guards. Genome Biol. 7, 212. https://doi.org/10.1186/gb-2006-7-4-212

Meister, G., 2013. Argonaute proteins: Functional insights and emerging roles. Nat. Rev. Genet. https://doi.org/10.1038/nrg3462

Méndez-Vigo, B., Gomaa, N.H., Alonso-Blanco, C., Xavier Picó, F., 2013. Among- and within-population variation in flowering time of Iberian *Arabidopsis thaliana* estimated in field and glasshouse conditions. New Phytol. 197, 1332–1343. https://doi.org/10.1111/nph.12082

Méndez-Vigo, B., Picó, F.X., Ramiro, M., Martínez-Zapater, J.M., Alonso-Blanco, C., 2011. Altitudinal and Climatic Adaptation Is Mediated by Flowering Traits and FRI, FLC, and PHYC Genes in Arabidopsis. Plant Physiol. 157, 1942–1955. https://doi.org/10.1104/PP.111.183426

Mérai, Z., Kerényi, Z., Molnár, A., Barta, E., Válóczi, A., Bisztray, G., Havelda, Z., Burgyán, J., Silhavy, D., 2005. Aureusvirus P14 is an efficient RNA silencing suppressor that binds double-stranded RNAs without size specificity. J. Virol. 79, 7217–26. https://doi.org/10.1128/JVI.79.11.7217-7226.2005

Mi, S., Cai, T., Hu, Y., Chen, Y., Hodges, E., Ni, F., Wu, L., Li, S., Zhou, H., Long, C., Chen, S., Hannon, G.J., Qi, Y., 2008. Sorting of Small RNAs into Arabidopsis Argonaute Complexes Is Directed by the 5′ Terminal Nucleotide. Cell 133, 116–127. https://doi.org/10.1016/j.cell.2008.02.034

Mingot, A., Valli, A., Rodamilans, B., San León, D., Baulcombe, D.C., García, J.A., López-Moya, J.J., 2016. The P1N-PISPO trans -Frame Gene of Sweet Potato Feathery Mottle Potyvirus Is Produced during Virus Infection and Functions as an RNA Silencing Suppressor . J. Virol. 90, 3543–3557. https://doi.org/10.1128/jvi.02360-15

Miyashita, Y., Atsumi, G., Nakahara, K.S., 2016. Trade-Offs for Viruses in Overcoming Innate Immunities in Plants. Mol. Plant-Microbe Interact. 29, 595–598. https://doi.org/10.1094/MPMI-05-16-0103-CR

Mlotshwa, S., Pruss, G.J., Vance, V., 2008. Small RNAs in viral infection and host defense. Trends Plant Sci. 13, 375–382. https://doi.org/10.1016/j.tplants.2008.04.009

Molnar, A., Melnyk, C.W., Bassett, A., Hardcastle, T.J., Dunn, R., Baulcombe, D.C., 2010. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science 328, 872–875. https://doi.org/10.1126/science.1187959

Montavon, T., Kwon, Y., Zimmermann, A., Hammann, P., Vincent, T., Cognat, V., Michel, F., Dunoyer, P., 2017. A specific dsRNA-binding protein complex selectively sequesters endogenous inverted-repeat siRNA precursors and inhibits their processing. Nucleic Acids Res. 45, 1330–1344. https://doi.org/10.1093/nar/gkw1264

Montes, N., Alonso-Blanco, C., García-Arenal, F., 2019. Cucumber mosaic virus infection as a potential selective pressure on Arabidopsis thaliana populations. PLOS Pathog. 15, e1007810. https://doi.org/10.1371/journal.ppat.1007810

Montgomery, T.A., Howell, M.D., Cuperus, J.T., Li, D., Hansen, J.E., Alexander, A.L., Chapman, E.J., Fahlgren, N., Allen, E., Carrington, J.C., 2008. Specificity of ARGONAUTE7-miR390 Interaction and Dual Functionality in TAS3 Trans-Acting siRNA Formation. Cell 133, 128–141. https://doi.org/10.1016/j.cell.2008.02.033

Morel, J., Godon, C., Mourrain, P., Béclin, C., Boutet, S., Feuerbach, F., Proux, F., Vaucheret, H., 2002. Fertile Hypomorphic *ARGONAUTE* (*ago1*) mutants impaired in post-transcriptional gene silencing and virus resistance. Society 14, 629–639. https://doi.org/10.1105/tpc.010358.are

Moreno, A.B., Martínez De Alba, A.E., Bardou, F., Crespi, M.D., Vaucheret, H., Maizel, A., Mallory, A.C., 2013. Cytoplasmic and nuclear quality control and turnover of single-stranded RNA modulate post-transcriptional gene silencing in plants. Nucleic Acids Res. 41, 4699–4708. https://doi.org/10.1093/nar/gkt152

Mourrain, P., Bé, C., Elmayan, T., Feuerbach, F., Godon, C., Morel, J.-B., Jouette, D., Lacombe, A.-M., Nikic, S., Picault, N., Ré, K., Sanial, M., Vo, T.-A., Vaucheret, H., 2000. Arabidopsis SGS2 and SGS3 Genes Are Required for Posttranscriptional Gene Silencing and Natural Virus Resistance gene required for PTGS, a possible mechanistic link. Cell 101,

533–542.

Moussian, B., Schoof, H., Haecker, A., Jurgens, G., Laux, T., 1998. Role of the ZWILLE gene in the regulation of central shoot meristem cell fate during Arabidopsis embryogenesis. EMBO J. 17, 1799–1809.

Muangsan, N., Beclin, C., Vaucheret, H., Robertson, D., 2004. Geminivirus VIGS of endogenous genes requires SGS2/SDE1 and SGS3 and defines a new branch in the genetic pathway for silencing in plants. Plant J. 38, 1004–1014. https://doi.org/10.1111/j.1365-313X.2004.02103.x

Mukherjee, K., Campos, H., Kolaczkowski, B., 2013. Evolution of animal and plant dicers: Early parallel duplications and recurrent adaptation of antiviral RNA binding in plants. Mol. Biol. Evol. 30, 627–641. https://doi.org/10.1093/molbev/mss263

Musiyenko, A., Majumdar, T., Andrews, J., Adams, B., Barik, S., 2012. PRMT1 methylates the single Argonaute of Toxoplasma gondii and is important for the recruitment of Tudor nuclease for target RNA cleavage by antisense guide RNA. Cell. Microbiol. 14, 882–901. https://doi.org/10.1111/j.1462-5822.2012.01763.x

Nakasugi, K., Crowhurst, R.N., Bally, J., Wood, C.C., Hellens, R.P., Waterhouse, P.M., 2013. De novo transcriptome sequence assembly and analysis of RNA silencing genes of Nicotiana benthamiana. PLoS One 8, e59534. https://doi.org/10.1371/journal.pone.0059534

Naylor, M., Murphy, A.M., Berry, J.O., Carr, J.P., 1998. Salicylic Acid Can Induce Resistance to Plant Virus Movement 11, 860–868.

Nguyen, H.D., Tomitaka, Y., Ho, S.Y.W., Duchêne, S., Vetten, H.-J., Lesemann, D., Walsh, J.A., Gibbs, A.J., Ohshima, K., 2013. Turnip Mosaic Potyvirus Probably First Spread to Eurasian Brassica Crops from Wild Orchids about 1000 Years Ago. PLoS One 8, e55336. https://doi.org/10.1371/journal.pone.0055336

Noah Fahlgren, T.A.M., Miya D. Howell,Edwards Allen, Sarah K. Dvorak, Amanda L. Alexander, and J.C.C., 2006. Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA Affects Developmental Timing and Patterning in Arabidopsis. Curr. Biol. 16, 939–944. https://doi.org/10.1016/j.cub.2006.03.065

Nonomura, K.-I., Morohoshi, A., Nakano, M., Eiguchi, M., Miyao, A., Hirochika, H., Kurata, N., 2007. A Germ Cell–Specific Gene of the ARGONAUTE Family Is Essential for the Progression of Premeiotic Mitosis and Meiosis during Sporogenesis in Rice. Plant Cell 19, 2583–2594. https://doi.org/10.1105/tpc.107.053199

Nordborg, M., Hu, T.T., Ishino, Y., Jhaveri, J., Toomajian, C., Zheng, H., Bakker, E., Calabrese, P., Gladstone, J., Goyal, R., Jakobsson, M., Kim, S., Morozov, Y., Padhukasahasram, B., Plagnol, V., Rosenberg, N.A., Shah, C., Wall, J.D., Wang, J., Zhao, K., Kalbfleisch, T., Schulz, V., Kreitman, M., Bergelson, J., 2005. The pattern of polymorphism in Arabidopsis thaliana. PLoS Biol. 3, 1289–1299. https://doi.org/10.1371/journal.pbio.0030196

Nuthikattu, S., McCue, A.D., Panda, K., Fultz, D., DeFraia, C., Thomas, E.N., Slotkin, R.K., 2013. The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21-22 nucleotide small interfering RNAs. Plant Physiol. 162, 116–31. https://doi.org/10.1104/pp.113.216481

Oa, W., Garcia-Ruiz, H., Takeda, A., Chapman, E.J., Sullivan, C.M., Fahlgren, N., Brempelis, K.J., Carrington, J.C., 2010. Arabidopsis RNA-Dependent RNA Polymerases and Dicer-Like Proteins in Antiviral Defense and Small Interfering RNA Biogenesis during Turnip Mosaic Virus Infection. Plant Cell 22, 481–496. https://doi.org/10.1105/tpc.109.073056

Obbard, D.J., Jiggins, F.M., Halligan, D.L., Little, T.J., 2006. Natural selection drives extremely rapid evolution in antiviral RNAi genes. Curr. Biol. 16, 580–585. https://doi.org/10.1016/j.cub.2006.01.065

Odokonyero, D., Mendoza, M.R., Alvarado, V.Y., Zhang, J., Wang, X., Scholthof, H.B., 2015. Transgenic down-regulation of ARGONAUTE2 expression in Nicotiana benthamiana interferes with several layers of antiviral defenses. Virology 486, 209–218. https://doi.org/10.1016/j.virol.2015.09.008

Odokonyero, D., Mendoza, M.R., Moffett, P., Scholthof, H.B., 2017. *Tobacco rattle virus* (TRV)-Mediated Silencing of *Nicotiana benthamiana ARGONAUTES* (*NbAGOs*) Reveals New Antiviral Candidates and Dominant Effects of TRV- *NbAGO1*. Phytopathology PHYTO-02-17-004. https://doi.org/10.1094/PHYTO-02-17-0049-R

Okano, Y., Senshu, H., Hashimoto, M., Neriya, Y., Netsu, O., Minato, N., Yoshida, T., Maejima, K., Oshima, K., Komatsu, K., Yamaji, Y., Namba, S., 2014. In Planta Recognition of a Double-Stranded RNA Synthesis Protein Complex by a Potexviral RNA Silencing Suppressor. Plant Cell 26, 2168–2183. https://doi.org/10.1105/tpc.113.120535

Olmedo-Monfil, V., Durán-Figueroa, N., Arteaga-Vázquez, M., Demesa-Arévalo, E., Autran, D., Grimanelli, D., Slotkin, R.K., Martienssen, R.A., Vielle-Calzada, J.-P., 2010. Control of female gamete formation by a small RNA pathway in Arabidopsis. Nature 464, 628–632. https://doi.org/10.1038/nature08828

Olspert, A., Chung, B.Y., Atkins, J.F., Carr, J.P., Firth, A.E., 2015. Transcriptional slippage in the positive-sense RNA virus family Potyviridae. EMBO Rep. 16, 995–1004. https://doi.org/10.15252/embr.201540509

Omarov, R.T., Ciomperlik, J., Scholthof, H.B., 2016. An in vitro reprogrammable antiviral RISC with size-preferential ribonuclease activity. Virology 490, 41–48. https://doi.org/10.1016/J.VIROL.2015.12.017

Pagán, I., Alonso-Blanco, C., García-Arenal, F., 2009. Differential Tolerance to Direct and Indirect Density-Dependent Costs of Viral Infection in Arabidopsis thaliana. PLoS Pathog. 5, e1000531. https://doi.org/10.1371/journal.ppat.1000531

Pagán, I., Alonso-Blanco, C., García-Arenal, F., 2008. Host Responses in Life-History Traits and Tolerance to Virus Infection in Arabidopsis thaliana. PLoS Pathog. 4, e1000124. https://doi.org/10.1371/journal.ppat.1000124

Pagán, I., Fraile, A., Fernandez-Fueyo, E., Montes, N., Alonso-Blanco, C., García-Arenal, F., 2010a. Arabidopsis thaliana as a model for the study of plant-virus co-evolution. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 365, 1983–95. https://doi.org/10.1098/rstb.2010.0062

Pagán, I., Fraile, A., Fernandez-Fueyo, E., Montes, N., Alonso-Blanco, C., García-Arenal, F., 2010b. Arabidopsis thaliana as a model for the study of plant–virus co-evolution. Philos. Trans. R. Soc. B Biol. Sci. 365, 1983–1995. https://doi.org/10.1098/rstb.2010.0062

Pagán, I., García-Arenal, F., 2018. Tolerance to Plant Pathogens: Theory and Experimental Evidence. Int. J. Mol. Sci. 19, 810. https://doi.org/10.3390/ijms19030810

Palukaitis, P., Roossinck, M.J., Dietzgen, R.G., Francki, R.I.B., 1992. Cucumber MOSAIC Virus. Adv. Virus Res. 41, 281–348. https://doi.org/10.1016/S0065-3527(08)60039-1

Parent, J.-S., Bouteiller, N., Elmayan, T., Vaucheret, H., 2015. Respective contributions of Arabidopsis DCL2 and DCL4 to RNA silencing. Plant J. 81, 223–232. https://doi.org/10.1111/tpj.12720

Paudel, D.B., Ghoshal, B., Jossey, S., Ludman, M., Fatyol, K., Sanfaçon, H., 2018. Expression and antiviral function of ARGONAUTE 2 in Nicotiana benthamiana plants infected with two isolates of tomato ringspot virus with varying degrees of virulence. Virology 524, 127–139. https://doi.org/10.1016/J.VIROL.2018.08.016

Paudel, D.B., Sanfaçon, H., 2018. Exploring the Diversity of Mechanisms Associated With Plant Tolerance to Virus Infection. Front. Plant Sci. 9, 1575. https://doi.org/10.3389/fpls.2018.01575

Paulo, J., Machado, B., Calil, I.P., Santos, A.A., Fontes, E.P.B., 2017. Translational control in plant antiviral immunity. Genet. Mol. Biol. 40, 292–304. https://doi.org/10.1590/1678-4685-GMB-2016-0092

Peart, J.R., Cook, G., Feys, B.J., Parker, J.E., Baulcombe, D.C., 2002a. An EDS1 orthologue is required for N-mediated resistance against tobacco mosaic virus. Plant J. 29, 569–579. https://doi.org/10.1046/j.1365-313X.2002.029005569.x

Peart, J.R., Lu, R., Sadanandom, A., Malcuit, I., Moffett, P., Brice, D.C., Schauser, L., Jaggard, D.A.W., Xiao, S., Coleman, M.J., Dow, M., Jones, J.D.G., Shirasu, K., Baulcombe, D.C., 2002b. Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. Proc. Natl. Acad. Sci. U. S. A. 99, 10865–10869. https://doi.org/10.1073/pnas.152330599

Picó, F.X., Méndez-Vigo, B., Martínez-Zapater, J.M., Alonso-Blanco, C., 2008. Natural Genetic Variation of Arabidopsis thaliana Is Geographically Structured in the Iberian Peninsula. Genetics 180, 1009–1021. https://doi.org/10.1534/genetics.108.089581

Pieterse, C.M.J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., Van Wees, S.C.M., 2012. Hormonal Modulation of Plant Immunity. Annu. Rev. Cell Dev. Biol. 28, 489–521.

https://doi.org/10.1146/annurev-cellbio-092910-154055

Pita, J.S., Morris, V., Roossinck, M.J., 2015. Mutation and Recombination Frequencies Reveal a Biological Contrast within Strains of Cucumber Mosaic Virus Downloaded from. J Virol 89, 6817–6823. https://doi.org/10.1128/JVI.01891-12

Pogany, J., Nagy, P.D., 2015. Activation of *Tomato Bushy Stunt Virus* RNA-Dependent RNA Polymerase by Cellular Heat Shock Protein 70 Is Enhanced by Phospholipids *In Vitro*. J. Virol. 89, 5714–5723. https://doi.org/10.1128/JVI.03711-14

Pogany, J., Nagy, P.D., 2012. p33-Independent Activation of a Truncated p92 RNA-Dependent RNA Polymerase of Tomato Bushy Stunt Virus in Yeast Cell-Free Extract. J. Virol. 86, 12025–12038.

Pontier, D., Picart, C., Roudier, F.O., Garcia, D., Lahmy, S., Azevedo, J., Alart, E., Le Laudié, M., Karlowski, W.M., Cooke, R., Colot, V., Voinnet, O., Lagrange, T., 2012. Molecular Cell Article NERD, a Plant-Specific GW Protein, Defines an Additional RNAi-Dependent Chromatin-Based Pathway in Arabidopsis. https://doi.org/10.1016/j.molcel.2012.07.027

Poulsen, C., Vaucheret, H., Brodersen, P., 2013. Lessons on RNA silencing mechanisms in plants from eukaryotic argonaute structures. Plant Cell 25, 22–37. https://doi.org/10.1105/tpc.112.105643

Powers, J.G., Sit, T.L., Qu, F., Morris, T.J., Kim, K.-H., Lommel, S.A., 2008. A Versatile Assay for the Identification of RNA Silencing Suppressors Based on Complementation of Viral Movement 21, 879–890.

Pradhan, M., Pandey, P., Gase, K., Sharaff, M., Singh, R., Sethi, A., Baldwin, I.T., Pandey, S.P., 2017. AGO8 mediates defense response against herbivores 2. Plant Physiol. https://doi.org/10.1104/pp.17.00702

Pumplin, N., Voinnet, O., 2013. RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence. Nat. Rev. Microbiol. 11, 745–60.

Qi, X., Bao, F.S., Xie, Z., 2009. Small RNA deep sequencing reveals role for Arabidopsis thaliana RNA-dependent RNA polymerases in viral siRNA biogenesis. PLoS One 4, e4971. https://doi.org/10.1371/journal.pone.0004971

Qi, Y., Denli, A.M., Hannon, G.J., 2005. Technique Biochemical Specialization within Arabidopsis RNA Silencing Pathways. Mol. Cell 19, 421–428. https://doi.org/10.1016/j.molcel.2005.06.014

Qu, F., Morris, T.J., 2005. Suppressors of RNA silencing encoded by plant viruses and their role in viral infections. FEBS Lett.

Qu, F., Ye, X., Morris, T.J., 2008. Arabidopsis DRB4, AGO1, AGO7, and RDR6 participate in a DCL4-initiated antiviral RNA silencing pathway negatively regulated by DCL1. Proc. Natl. Acad. Sci. U. S. A. 105, 14732–7. https://doi.org/10.1073/pnas.0805760105

Raja, Priya, Jackel, J.N., Li, S., Heard, I.M., Bisaro, D.M., 2014. Arabidopsis double-stranded RNA binding protein DRB3 participates in methylation-mediated defense against geminiviruses. J. Virol. 88, 2611–22. https://doi.org/10.1128/JVI.02305-13

Raja, P., Jackel, J.N., Li, S., Heard, I.M., Bisaro, D.M., 2014. Arabidopsis Double-Stranded RNA Binding Protein DRB3 Participates in Methylation-Mediated Defense against Geminiviruses. J. Virol. 88, 2611–2622. https://doi.org/10.1128/JVI.02305-13

Raja, P., Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008a. Viral Genome Methylation as an Epigenetic Defense against Geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, Priya, Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008. Viral genome methylation as an epigenetic defense against geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, P., Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008b. Viral Genome Methylation as an Epigenetic Defense against Geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, P., Wolf, J.N., Bisaro, D.M., 2010. RNA silencing directed against geminiviruses: Post-transcriptional and epigenetic components. Biochim. Biophys. Acta - Gene Regul. Mech. 1799, 337–351. https://doi.org/10.1016/j.bbagrm.2010.01.004

Rambod Abiri, al, Valdiani, A., Maziah, M., Azmi Shaharuddin, N., Sahebi, M., Norhana Balia Yusof, Z., Atabaki, N., Talei, D., Valdiani alireza, A., 2016. A Critical Review of the

Concept of Transgenic Plants: Insights into Pharmaceutical Biotechnology and Molecular Farming. Curr. Issues Mol. Biol. 18, 21–42. https://doi.org/10.21775/cimb.018.021

Reinhart, B.J., Weinstein, E.G., Rhoades, M.W., Bartel, B., Bartel, D.P., 2002. MicroRNAs in plants. Genes Dev.

Revers, F., García, J.A., 2015a. Molecular biology of potyviruses, in: Advances in Virus Research. Academic Press Inc., pp. 101–199. https://doi.org/10.1016/bs.aivir.2014.11.006

Revers, F., García, J.A., 2015b. Molecular Biology of Potyviruses. Adv. Virus Res. 92, 101–199. https://doi.org/10.1016/BS.AIVIR.2014.11.006

Reyero-Saavedra, M. del R., Qiao, Z., Sánchez-Correa, M. del S., Reyes, J.L., Covarrubias, A.A., Libault, M., Valdés-López, O., 2017. Gene Silencing of Argonaute5 Negatively Affects the Establishment of the Legume-Rhizobia Symbiosis. Genes (Basel). 5, 1–19. https://doi.org/10.3390/genes8120352

Rivas, F. V, Tolia, N.H., Song, J.-J., Aragon, J.P., Liu, J., Hannon, G.J., Joshua-Tor, L., 2005. Purified Argonaute2 and an siRNA form recombinant human RISC. Nat. Struct. Mol. Biol. 12, 340–349. https://doi.org/10.1038/nsmb918

Rodríguez-Leal, D., Castillo-Cobián, A., Rodríguez-Arévalo, I., Vielle-Calzada, J.-P., 2016. A Primary Sequence Analysis of the ARGONAUTE Protein Family in Plants. Front. Plant Sci. 7, 1347. https://doi.org/10.3389/fpls.2016.01347

Roossinck, M.J., 2002. Evolutionary history of Cucumber mosaic virus deduced by phylogenetic analyses. J. Virol. 76, 3382–7. https://doi.org/10.1128/jvi.76.7.3382-3387.2002

Roossinck, M.J., 2001. Cucumber mosaic virus, a model for RNA virus evolution. Mol. Plant Pathol. 2, 59–63. https://doi.org/10.1046/j.1364-3703.2001.00058.x

Rosa, C., Kuo, Y.-W., Wuriyanghan, H., Falk, B.W., 2018. RNA Interference Mechanisms and Applications in Plant Pathology. Annu. Rev. Phytopathol. 56, 581–610. https://doi.org/10.1146/annurev-phyto-080417

Rose, L., Atwell, S., Grant, M., Holub, E.B., 2012. Parallel Loss-of-Function at the RPM1 Bacterial Resistance Locus in Arabidopsis thaliana. Front. Plant Sci. 3, 287.

https://doi.org/10.3389/fpls.2012.00287

Roth, B.M., Pruss, G.J., Vance, V.B., 2004. Review-Plant viral suppressors of RNA silencing. Virus Res. 102, 97–108. https://doi.org/10.1016/j.virusres.2004.01.020

Sanjuán, R., Nebot, M.R., Chirico, N., Louis, M., Belshaw, R., Sanjua, R., Mansky, L.M., 2010. Viral Mutation Rates Viral Mutation Rates □. J. Virol. 84, 9733–9748. https://doi.org/10.1128/JVI.00694-10

Saumet, A., Lecellier, C.-H., 2006. Anti-viral RNA silencing: do we look like plants? Retrovirology 3, 3. https://doi.org/10.1186/1742-4690-3-3

Schepetilnikov, M. V., Manske, U., Solovyev, A.G., Jr, A.A.Z., Schiemann, J., Morozov, S.Y., 2005. The hydrophobic segment of Potato virus X TGBp3 is a major determinant of the protein intracellular trafficking. J. Gen. Virol. 86, 2379–2391. https://doi.org/10.1099/vir.0.80865-0

Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Schölkopf, B., Weigel, D., Lohmann, J.U., 2005. A gene expression map of Arabidopsis thaliana development. Nat. Genet. 37, 501–506. https://doi.org/10.1038/ng1543

Scholthof, H.B., Alvarado, V.Y., Vega-Arreguin, J.C., Ciomperlik, J., Odokonyero, D., Brosseau, C., Jaubert, M., Zamora, A., Moffett, P., 2011. Identification of an ARGONAUTE for antiviral RNA silencing in Nicotiana benthamiana. Plant Physiol. 156, 1548–55. https://doi.org/10.1104/pp.111.178764

Schott, G., Mari-Ordonez, A., Himber, C., Alioua, A., Voinnet, O., Dunoyer, P., 2012. Differential effects of viral silencing suppressors on siRNA and miRNA loading support the existence of two distinct cellular pools of ARGONAUTE1. EMBO J. 31, 2553–65. https://doi.org/10.1038/emboj.2012.92

Schuck, J., Gursinsky, T., Pantaleo, V., Burgy??n, J., Behrens, S.E., 2013a. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic Acids Res. 41, 5090–5103.

Schuck, J., Gursinsky, T., Pantaleo, V., Burgyán, J., Behrens, S.-E., 2013b. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic Acids Res. 41, 5090–103.

https://doi.org/10.1093/nar/gkt193

Senshu, H., Ozeki, J., Komatsu, K., Hashimoto, M., Hatada, K., Aoyama, M., Kagiwada, S., Yamaji, Y., Namba, S., 2017. Variability in the level of RNA silencing suppression caused by triple gene block protein 1 (TGBp1) from various potexviruses during infection. https://doi.org/10.1099/vir.0.008243-0

Senshu, H., Ozeki, J., Komatsu, K., Hashimoto, M., Hatada, K., Aoyama, M., Kagiwada, S., Yamaji, Y., Namba, S., 2009. Variability in the level of RNA silencing suppression caused by triple gene block protein 1 (TGBp1) from various potexviruses during infection. J. Gen. Virol. https://doi.org/10.1099/vir.0.008243-0

Senthil-Kumar, M., Mysore, K.S., 2013. Nonhost Resistance Against Bacterial Pathogens: Retrospectives and Prospects. Annu. Rev. Phytopathol. https://doi.org/10.1146/annurev-phyto-082712-102319

Shamandi, N., Zytnicki, M., Charbonnel, C., Elvira-Matelot, E., Bochnakian, A., Comella, P., Mallory, A.C., Lepère, G., Sáez-Vásquez, J., Vaucheret, H., 2015a. Plants Encode a General siRNA Suppressor That Is Induced and Suppressed by Viruses. PLoS Biol. 13.

Shamandi, N., Zytnicki, M., Charbonnel, C., Elvira-Matelot, E., Bochnakian, A., Comella, P., Mallory, A.C., Lepère, G., Sáez-Vásquez, J., Vaucheret, H., 2015b. Plants Encode a General siRNA Suppressor That Is Induced and Suppressed by Viruses. PLOS Biol. 13, e1002326. https://doi.org/10.1371/journal.pbio.1002326

Shen, Q., Hu, T., Bao, M., Cao, L., Zhang, H., Song, F., Xie, Q., Zhou, X., 2016. Tobacco RING E3 Ligase NtRFP1 Mediates Ubiquitination and Proteasomal Degradation of a Geminivirus-Encoded βC1. Mol. Plant 9, 911–925. https://doi.org/10.1016/j.molp.2016.03.008

Shukla, A., Pagán, I., García-Arenal, F., 2018. Effective tolerance based on resource reallocation is a virus-specific defence in *Arabidopsis thaliana*. Mol. Plant Pathol. 19, 1454–1465. https://doi.org/10.1111/mpp.12629

Silhavy, D., Molnár, A., Lucioli, A., Szittya, G., Hornyik, C., Tavazza, M., Burgyán, J., 2002. A viral protein suppresses RNA silencing and binds silencing-generated, 21- to 25-nucleotide double-stranded RNAs. EMBO J. 21, 3070–80. https://doi.org/10.1093/emboj/cdf312

Siomi, M.C., Mannen, T., Siomi, H., 2010. How does the royal family of tudor rule the PIWI-interacting RNA pathway? Genes Dev. 24, 636–646. https://doi.org/10.1101/gad.1899210

Solovyev, A.G., Kalinina, N.O., Morozov, S.Y., 2012. Recent Advances in Research of Plant Virus Movement Mediated by Triple Gene Block. Front. Plant Sci. https://doi.org/10.3389/fpls.2012.00276

Solovyev, A.G., Stroganova, T.A., Zamyatnin, A.A., Fedorkin, O.N., Schiemann, J., Morozov, S.Y., 2000. Subcellular Sorting of Small Membrane-Associated Triple Gene Block Proteins: TGBp3-Assisted Targeting of TGBp2. Virology 269, 113–127. https://doi.org/10.1006/VIRO.2000.0200

Song, J.-J., Smith, S.K., HannonL, G.J., Joshua-Tor, L., 2004. Crystal Structure of Argonaute and Its Implications for RISC Slicer Activity. Science (80-.). 305, 1434–1437. https://doi.org/10.1126/science.1102514

Song, M.-S., Rossi, J.J., 2017. Molecular mechanisms of Dicer: endonuclease and enzymatic activity. Biochem. J. 474, 1603–1618. https://doi.org/10.1042/bcj20160759

Stael, S., Kmiecik, P., Willems, P., Van Der Kelen, K., Coll, N.S., Teige, M., Van Breusegem, F., 2015. Plant innate immunity--sunny side up? Trends Plant Sci. 20, 3–11. https://doi.org/10.1016/j.tplants.2014.10.002

Stahl, E.A., Dwyer, G., Mauricio, R., Kreitman, M., Bergelson, J., 1999. Dynamics of disease resistance polymorphism at the Rpm1 locus of Arabidopsis. Nature 400, 667–671. https://doi.org/10.1038/23260

Sun, Y., Sparks, C., Jones, H., Riley, M., Francis, F., Du, W., Xia, L., 2019. Silencing an essential gene involved in infestation and digestion in grain aphid through plant-mediated interference generates aphid-resistant wheat plants. Plant Biotechnol. J. 17, 852–854. https://doi.org/10.1111/pbi.13067

Syller, J., 2012. Facilitative and antagonistic interactions between plant viruses in mixed infections. Mol. Plant Pathol. https://doi.org/10.1111/j.1364-3703.2011.00734.x

Szittya, G., Burgyán, J., 2013. RNA Interference-Mediated Intrinsic Antiviral Immunity in Plants. https://doi.org/10.1007/978-3-642-37765-5_6

Szittya, G., Moxon, S., Pantaleo, V., Toth, G., Rusholme Pilcher, R.L., Moulton, V., Burgyan, J., Dalmay, T., 2010. Structural and functional analysis of viral siRNAs. PLoS Pathog. 6, e1000838. https://doi.org/10.1371/journal.ppat.1000838

Takahashi, H., Miller, J., Nozaki, Y., Takeda, M., Shah, J., Hase, S., Ikegami, M., Ehara, Y., Dinesh-Kumar, S.P., 2002. RCY1, an Arabidopsis thaliana RPP8/HRT family resistance gene, conferring resistance to cucumber mosaic virus requires salicylic acid, ethylene and a novel signal transduction mechanism. Plant J. 32, 655–667. https://doi.org/10.1046/j.1365-313X.2002.01453.x

Takahashi, H., Suzuki, M., Natsuaki, K., Shigyo, T., Hino, K., Teraoka, T., Hosokawa, D., Ehara, Y., 2001. Mapping the virus and host genes involved in the resistance response in cucumber mosaic virus-Infected Arabidopsis thaliana. Plant Cell Physiol. 42, 340–7.

Takeda, A., Iwasaki, S., Watanabe, T., Utsumi, M., Watanabe, Y., 2008. The Mechanism Selecting the Guide Strand from Small RNA Duplexes is Different Among Argonaute Proteins. Plant Cell Physiol. 49, 493–500. https://doi.org/10.1093/pcp/pcn043

Tecson Mendoza, E.M., C. Laurena, A., Botella, J.R., 2008. Recent advances in the development of transgenic papaya technology. Biotechnol. Annu. Rev. 14, 423–462. https://doi.org/10.1016/S1387-2656(08)00019-7

Thomas-Sharma, S., Abdurahman, A., Ali, S., Andrade-Piedra, J.L., Bao, S., Charkowski, A.O., Crook, D., Kadian, M., Kromann, P., Struik, P.C., Torrance, L., Garrett, K.A., Forbes, G.A., 2016. Seed degeneration in potato: the need for an integrated seed health strategy to mitigate the problem in developing countries. Plant Pathol. 65, 3–16. https://doi.org/10.1111/ppa.12439

Thompson, K., Stewart, A.J.A., 1981. The Measurement and Meaning of Reproductive Effort in Plants. Am. Nat. 117, 205–211. https://doi.org/10.1086/283700

Tilsner, J., Linnik, O., Wright, K.M., Bell, K., Roberts, A.G., Lacomme, C., Santa Cruz, S., Oparka, K.J., 2012. The TGB1 movement protein of Potato virus X reorganizes actin and endomembranes into the X-body, a viral replication factory. Plant Physiol. 158, 1359–70. https://doi.org/10.1104/pp.111.189605

Todesco, M., Balasubramanian, S., Hu, T.T., Traw, M.B., Horton, M., Epple, P., Kuhns, C., Sureshkumar, S., Schwartz, C., Lanz, C., Laitinen, R.A.E., Huang, Y., Chory, J., Lipka, V., Borevitz, J.O., Dangl, J.L., Bergelson, J., Nordborg, M., Weigel, D., 2010. Natural allelic variation underlying a major fitness trade-off in Arabidopsis thaliana. Nature 465. https://doi.org/10.1038/nature09083

Törjék, O., Meyer, R.C., Zehnsdorf, M., Teltow, M., Strompen, G., Witucka-Wall, H., Blacha, A., Altmann, T., 2008. Construction and Analysis of 2 Reciprocal Arabidopsis Introgression Line Populations. J. Hered. 99, 396–406. https://doi.org/10.1093/jhered/esn014

Tsuzuki, M., Takeda, A., Watanabe, Y., 2014. Recovery of dicer-like 1 -late flowering phenotype by miR172 expressed by the noncanonical DCL4-dependent biogenesis pathway. RNA 20, 1320–1327. https://doi.org/10.1261/rna.044966.114

Tucker, M.R., Okada, T., Hu, Y., Scholefield, a., Taylor, J.M., Koltunow, a. M.G., 2012. Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in Arabidopsis. Development 139, 1399–1404. https://doi.org/10.1242/dev.075390

Vagin, V. V, Wohlschlegel, J., Qu, J., Jonsson, Z., Huang, X., Chuma, S., Girard, A., Sachidanandam, R., Hannon, G.J., Aravin, A.A., 2009. methylation in specifying interaction with Tudor family members Proteomic analysis of murine Piwi proteins reveals a role for arginine methylation in specifying interaction with Tudor family members 1749–1762. https://doi.org/10.1101/gad.1814809

Vance, V., 2001. RNA Silencing in Plants--Defense and Counterdefense. Science (80-.). 292, 2277–2280. https://doi.org/10.1126/science.1061334

Várallyay, É., Oláh, E., Havelda, Z., 2014. Independent parallel functions of p19 plant viral suppressor of RNA silencing required for effective suppressor activity. Nucleic Acids Res. 42, 599–608. https://doi.org/10.1093/nar/gkt846

Varma, A. and M.N., 2001. Durable host-plant resistance, a desirable trait for integrated disease management.

Varsha Wesley, S., Helliwell, C.A., Smith, N.A., Wang, M., Rouse, D.T., Liu, Q., Gooding, P.S.,

Singh, S.P., Abbott, D., Stoutjesdijk, P.A., Robinson, S.P., Gleave, A.P., Green, A.G., Waterhouse, P.M., 2001. Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J. 27, 581–590. https://doi.org/10.1046/j.1365-313X.2001.01105.x

Vaucheret, H., 2008. Plant ARGONAUTES. Trends Plant Sci.

Vaucheret, H., Mallory, A.C., Bartel, D.P., 2006. AGO1 Homeostasis Entails Coexpression of MIR168 and AGO1 and Preferential Stabilization of miR168 by AGO1. Mol. Cell 22, 129–136.

Vaucheret, H., Vazquez, F., Crété, P., Bartel, D.P., 2004. The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. Genes Dev. 18, 1187–1197. https://doi.org/10.1101/gad.1201404

Verchot-Lubicz, J., Torrance, L., Solovyev, A.G., Morozov, S.Y., Jackson, A.O., Gilmer, D., 2010. Varied Movement Strategies Employed by Triple Gene Block–Encoding Viruses 23, 1231–1247. https://doi.org/10.1094

Verlaan, M.G., Hutton, S.F., Ibrahem, R.M., Kormelink, R., Visser, R.G.F., Scott, J.W., Edwards, J.D., Bai, Y., 2013. The Tomato Yellow Leaf Curl Virus Resistance Genes Ty-1 and Ty-3 Are Allelic and Code for DFDGD-Class RNA-Dependent RNA Polymerases. PLoS Genet. 9.

Vickers, T.A., Koo, S., Bennett, C.F., Crooke, S.T., Dean, N.M., Baker, B.F., 2003. Efficient reduction of target RNAs by small interfering RNA and RNase H-dependent antisense agents. A comparative analysis. J. Biol. Chem. 278, 7108–7118.

Voinnet, O., Lederer, C., Baulcombe, D.C., 2000. A Viral Movement Protein Prevents Spread of the Gene Silencing Signal in Nicotiana benthamiana. Cell 103, 157–167. https://doi.org/10.1016/S0092-8674(00)00095-7

Voinnet, O., Pinto, Y.M., Baulcombe, D.C., Lohuis, D., Visser, R.G.F., Bai, Y., Kormelink, R., 1999. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. Proc. Natl. Acad. Sci. U. S. A. 96, 14147–52. https://doi.org/10.1073/pnas.96.24.14147

Wang, D., Liu, Q., Jones, H.D., Bruce, T., Xia, L., 2014. Comparative transcriptomic analyses

revealed divergences of two agriculturally important aphid species. BMC Genomics 15. https://doi.org/10.1186/1471-2164-15-1023

Wang, J., Mei, J., Ren, G., 2019. Plant microRNAs: Biogenesis, Homeostasis, and Degradation. Front. Plant Sci. 10, 360. https://doi.org/10.3389/fpls.2019.00360

Wang, M.-B., Metzlaff, M., 2005. RNA silencing and antiviral defense in plants. Curr. Opin. Plant Biol. 8, 216–222. https://doi.org/10.1016/j.pbi.2005.01.006

Wang, X.-B., Jovel, J., Udomporn, P., Wang, Y., Wu, Q., Li, W.-X., Gasciolli, V., Vaucheret, H., Ding, S.-W., 2011. The 21-Nucleotide, but Not 22-Nucleotide, Viral Secondary Small Interfering RNAs Direct Potent Antiviral Defense by Two Cooperative Argonautes in Arabidopsis thaliana. Plant Cell 23, 1625–1638. https://doi.org/10.1105/tpc.110.082305

Wang, X.-B., Wu, Q., Ito, T., Cillo, F., Li, W.-X., Chen, X., Yu, J.-L., Ding, S.-W., 2010. RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 107, 484–489. https://doi.org/10.1073/pnas.0904086107

Wassenegger, M., Krczal, G., 2006. Nomenclature and functions of RNA-directed RNA polymerases. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2006.01.003

Wei, T., Wang, L., Zhou, X., Ren, X., Dai, X., Liu, H., 2015. PopW activates PAMP-triggered immunity in controlling tomato bacterial spot disease. Biochem. Biophys. Res. Commun. 463, 746–750.

Wei, W., Ba, Z., Gao, M., Wu, Y., Ma, Y., Amiard, S., White, C.I., Rendtlew Danielsen, J.M., Yang, Y.-G., Qi, Y., 2012. A Role for Small RNAs in DNA Double-Strand Break Repair. Cell 149, 101–112. https://doi.org/10.1016/j.cell.2012.03.002

Willmann, M.R., Endres, M.W., Cook, R.T., Gregory, B.D., 2011. The Functions of RNA-Dependent RNA Polymerases in Arabidopsis. Arab. B. 9, e0146. https://doi.org/10.1199/tab.0146

Wu, J., Yang, Z., Wang, Y., Zheng, L., Ye, R., Ji, Y., Zhao, S., Ji, S., Liu, R., Xu, L., Zheng, H., Zhou, Y., Zhang, X., Cao, X., Xie, L., Wu, Z., Qi, Y., Li, Y., 2015. Viral-inducible Argonaute18 confers broad-spectrum virus resistance in rice by sequestering a host

microRNA. Elife 4. https://doi.org/10.7554/eLife.05733

Xiao, Y., Wu, K., 2019. Recent progress on the interaction between insects and Bacillus thuringiensis crops. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2018.0316

Xie, Z., Johansen, L.K., Gustafson, A.M., Kasschau, K.D., Lellis, A.D., Zilberman, D., Jacobsen, S.E., Carrington, J.C., 2004. Genetic and functional diversification of small RNA pathways in plants. PLoS Biol. 2, E104. https://doi.org/10.1371/journal.pbio.0020104

Xu, D., Zhou, G., 2017. Characteristics of siRNAs derived from Southern rice black-streaked dwarf virus in infected rice and their potential role in host gene regulation. Virol. J. 14, 27. https://doi.org/10.1186/s12985-017-0699-3

Yamaji, Y., Maejima, K., Ozeki, J., Komatsu, K., Shiraishi, T., Okano, Y., Himeno, M., Sugawara, K., Neriya, Y., Minato, N., Miura, C., Hashimoto, M., Namba, S., 2012. Lectin-mediated resistance impairs plant virus infection at the cellular level. Plant Cell 24, 778–93. https://doi.org/10.1105/tpc.111.093658

Yang, L., Li, B., Zheng, X.Y., Li, J., Yang, M., Dong, X., He, G., An, C., Deng, X.W., 2015. Salicylic acid biosynthesis is enhanced and contributes to increased biotrophic pathogen resistance in Arabidopsis hybrids. Nat. Commun. 6, 1–11. https://doi.org/10.1038/ncomms8309

Ye, J., Yang, J., Sun, Y., Zhao, P., Gao, S., Jung, C., Qu, J., Fang, R., Chua, N.H., 2015. Geminivirus Activates ASYMMETRIC LEAVES 2 to Accelerate Cytoplasmic DCP2-Mediated mRNA Turnover and Weakens RNA Silencing in Arabidopsis. PLoS Pathog. 11.

Ye, R., Wang, W., Iki, T., Liu, C., Wu, Y., Ishikawa, M., Zhou, X., Qi, Y., 2012. Cytoplasmic Assembly and Selective Nuclear Import of Arabidopsis ARGONAUTE4/siRNA Complexes. Mol. Cell 46, 859–870. https://doi.org/10.1016/j.molcel.2012.04.013

Yoshikawa, M., 2013. Biogenesis of trans-acting siRNAs, endogenous secondary siRNAs in plants. Genes Genet. Syst. 88, 77–84.

Yoshikawa, M., Iki, T., Tsutsui, Y., Miyashita, K., Poethig, R.S., Habu, Y., Ishikawa, M., 2013. 3′ fragment of miR173-programmed RISC-cleaved RNA is protected from degradation in a

complex with RISC and SGS3. PNAS 110, 4117–4122. https://doi.org/10.1073/pnas.1217050110

Yu, X.D., Liu, Z.C., Huang, S.L., Chen, Z.Q., Sun, Y.W., Duan, P.F., Ma, Y.Z., Xia, L.Q., 2016. RNAi-mediated plant protection against aphids. Pest Manag. Sci.

Zhang, G., Chen, M., Li, L., Xu, Z., Chen, X., Guo, J., Ma, Y., 2009. Overexpression of the soybean GmERF3 gene, an AP2/ERF type transcription factor for increased tolerances to salt, drought, and diseases in transgenic tobacco. J. Exp. Bot. 60, 3781–96. https://doi.org/10.1093/jxb/erp214

Zhang, H., Xia, R., Walbot, V., 2015. Evolution, functions, and mysteries of plant ARGONAUTE proteins. Curr. Opin. Plant Biol. 27, 84–90. https://doi.org/10.1016/J.PBI.2015.06.011

Zhang, W., Sun, X., Yuan, H., Araki, H., Wang, J., Tian, D., 2008. The pattern of insertion/deletion polymorphism in Arabidopsis thaliana. Mol. Genet. Genomics 280, 351–361. https://doi.org/10.1007/s00438-008-0370-1

Zhang, Xiaoming, Xia, J., Lii, Y., Barrera-Figueroa, B., Zhou, X., Gao, S., Lu, L., Niu, D., Liang, W., Chen, Z., Leung, C., Wong, T., Guo, J., Li, Y., Liu, R., Zhu, J.-K., Zhang, W., Jin, H., Zhang, H., 2012. Genome-wide analysis of plant nat-siRNAs reveals insights into their distribution, biogenesis and function. Genome Biol. 13, R20.

Zhang, X., Yuan, Y.-R., Pei, Y., Lin, S.-S., Tuschl, T., Patel, D.J., Chua, N.-H., 2006. Cucumber mosaic virus-encoded 2b suppressor inhibits Arabidopsis Argonaute1 cleavage activity to counter plant defense. Genes Dev. 20, 3255–68. https://doi.org/10.1101/gad.1495506

Zhang, Xiaofeng, Zhang, X., Singh, J., Li, D., Qu, F., 2012. Temperature-Dependent Survival of Turnip Crinkle Virus-Infected Arabidopsis Plants Relies on an RNA Silencing-Based Defense That Requires DCL2, AGO2, and HEN1. J. Virol. 86, 6847–6854. https://doi.org/10.1128/JVI.00497-12

Zhang, X., Zhao, H., Gao, S., Wang, W.-C., Katiyar-Agarwal, S., Huang, H.-D., Raikhel, N., Jin, H., 2011. Arabidopsis Argonaute 2 Regulates Innate Immunity via miRNA393∗-Mediated Silencing of a Golgi-Localized SNARE Gene, MEMB12. Mol. Cell 42, 356–366.

https://doi.org/10.1016/j.molcel.2011.04.010

Zhang, Xiuchun, Zhang, Xiaofeng, Singh, J., Li, D., Qu, F., 2012. Temperature-dependent survival of Turnip crinkle virus-infected arabidopsis plants relies on an RNA silencing-based defense that requires dcl2, AGO2, and HEN1. J. Virol. 86, 6847–54. https://doi.org/10.1128/JVI.00497-12

Zhang, Z., Liu, X., Guo, X., Wang, X.-J., Zhang, X., 2016. Arabidopsis AGO3 predominantly recruits 24-nt small RNAs to regulate epigenetic silencing. Nat. Plants 2, 16049. https://doi.org/10.1038/nplants.2016.49

Zhao, K., Zhao, H., Chen, Z., Feng, L., Ren, J., Cai, R., Xiang, Y., 2015. The Dicer-like, Argonaute and RNA-dependent RNA polymerase gene families in Populus trichocarpa: gene structure, gene expression, phylogenetic analysis and evolution. J. Genet. 94, 317–321. https://doi.org/10.1007/s12041-015-0508-y

Zhao, Y., Chang, X., Qi, D., Dong, L., Wang, G., Fan, S., Jiang, L., Cheng, Q., Chen, X., Han, D., Xu, P., Zhang, S., 2017. A Novel Soybean ERF Transcription Factor, GmERF113, Increases Resistance to Phytophthora sojae Infection in Soybean. Front. Plant Sci. 8, 299. https://doi.org/10.3389/fpls.2017.00299

Zheng, X., Fahlgren, N., Abbasi, A., Berry, J.C., Carrington, J.C., 2019a. Antiviral ARGONAUTEs Against Turnip Crinkle Virus Revealed by Image-Based Trait Analysis. Plant Physiol. 180, 1418–1435. https://doi.org/10.1104/pp.19.00121

Zheng, X., Fahlgren, N., Abbasi, A., Berry, J.C., Carrington, J.C., 2019b. Antiviral ARGONAUTEs Against Turnip Crinkle Virus Revealed by Image-Based Trait Analysis. Plant Physiol. 180, 1418–1435. https://doi.org/10.1104/pp.19.00121

Zheng, X., Zhu, J., Kapoor, A., Zhu, J.-K., Liu, J., Chen, X., Harmer, S., Zilberman, D., Pikaard, C., Watson, J., Rezaian, A., Dennis, E., Waterhouse, P., Martienssen, R., 2007. Role of Arabidopsis AGO6 in siRNA accumulation, DNA methylation and transcriptional gene silencing. EMBO J. 26, 1691–701. https://doi.org/10.1038/sj.emboj.7601603

Zhou, X.-F., Sun, J.-D., Zhuo, Z., Jie, L. V, Wei, X.-W., Rui, C., Xu, H.-W., 2012. The Feasibility Analysis of PVX and TRV Vectors as the VIGS Tool for Studying the Gene

Function peer-review under responsibility of [name organizer]. Int. Conf. Med. Phys. Biomed. Eng. 33, 46–54. https://doi.org/10.1016/j.phpro.2012.05.029

Zhou, Y., Honda, M., Duan, L., Zhang Correspondence, X., Zhu, H., Zhang, Z., Guo, X., Li, T., Li, Z., Peng, X., Nakajima, K., Zhang, X., 2015. Spatiotemporal Sequestration of miR165/166 by Arabidopsis Argonaute10 Promotes Shoot Apical Meristem Maintenance. CellReports 10, 1819–1827. https://doi.org/10.1016/j.celrep.2015.02.047

Zhu, H., Hu, F., Wang, R., Zhou, X., Sze, S.-H., Liou, L.W., Barefoot, A., Dickman, M., Zhang, X., Qi, Y, Chen, S., Hannon, G., Qi, Y, Chen, X., Chen, X., Wu, Z., Qi, Yijun, Li, Y., 2011. Arabidopsis Argonaute10 Specifically Sequesters miR166/165 to Regulate Shoot Apical Meristem Development. Cell 145, 242–256. https://doi.org/10.1016/j.cell.2011.03.024

Zilberman, D., Cao, X., Jacobsen, S.E., 2003. ARGONAUTE4 control of locus-specific siRNA accumulation and DNA and histone methylation. Science 299, 716–9. https://doi.org/10.1126/science.1079695

Zust, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., Barchet, W., Diamond, M.S., Siddell, S.G., Ludewig, B., Thiel, V., 2011. Ribose 2'-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. Nat Immunol 12, 137–143.

3.7 Supplemental data

3.7.1 Supplementary Figure 1:

Figure S3.1: Screening of *N. benthamiana* and tomato plants for the presence of AtAGO2 and NbAGO2. Samples were harvested pre-infection and subjected to immune blotting with anti-HA antibody.

3.7.2 Table 3.1: Primers used in this study.

NbProm-Fwd	ggtaccCTATTTGCTCTTGTTCTTGAG
NbProm-Rev	tctagaTTTCCAACTTCACTAAAGAG
NbAGO2TermFwd	ggatcccccgggCTATTTGCTCTTGTTCTTGAG
NbAGO2TermRev	cctgcaggTTCGCTATTTCTGCTGGAGTTAC
HA-AtAGO2-Fwd	TCTAGAGCCACCATGTACCCATACGATGTTCCTGACTATGCGATGGAGAG AGGTGGTTATCG
AtAGO2-Rev	GGATCCTCAGACGAAGAACATAACATTCTC
HA-NbAGO2-Fwd	CCTAGGATGGACTACAAAGACGATGATGACAAGTCTAGAATGGATCGTGG AAATTACC
HA-NbAGO2-Rev	GGATCCTCAGACAAAGAACATTATGTTC

CHAPTER 4

4. DISCUSSION AND GENERAL CONCLUSION

4.1 Variation in *AGO2*: comparison with Natural variation in other viral defense-related genes.

The role of RNA silencing in plant pathogen defense has been established in several plant-viruses interaction (Carbonell and Carrington, 2015). Work presented here seem to indicate that natural variations in *AGO2* gene may serve as a means of defense for plants in wild populations. By transiently expressing the AGO proteins of *A. thaliana*, it has been reported that all *A. thaliana* AGO proteins possess the ability to inherently target viral RNA that is not protected by a VSR (Brosseau and Moffett 2015). However, for a given AGO to function in antiviral resistance, it can be proposed that it needs to be expressed in the right place and at the right time. This would account for the minor role, or lack thereof for AGO proteins with developmentally or spatially restricted expression patterns, such as AGO6 or AGO10, which contrasts with the constitutive and ubiquitous pattern of AGO2 (Silva-Martins, Adurogbangba and Moffett, in press). Over the years, questions such as: (1) what is the mechanisms that regulate endogenous RNA silencing gene expression during virus infection and (2) why different studies identify different AGO proteins effecting defense for different viruses have been answered in part. However, the full impact of genetic variations in AGO proteins has not been well documented. The projects presented in chapters 2 and 3 are yet to be reported elsewhere and they explore the implications that these genetic variations have on viral resistance in naturally occurring plant populations.

There are several examples in the literature demonstrating that variability in single plant genes can affect the resistance / susceptibility phenotype to various pathogens, including viruses. A well-studied example of genetic diversity in resistance is the restricted TEV movement (RTM)

resistance gene. These genes restrict the long-distance movement of several potyviruses (Decroocq et al., 2009; Mahajan et al., 1998). The genetic characterization of natural *A. thaliana* accessions of the *RTM* gene showed that a single mutation in one of the *RTM* genes abolishes this resistance (Cosson et al., 2012, 2010; Decroocq et al., 2009). Studies of the *JAX1-JACALIN-TYPE LECTIN REQUIRED FOR POTEXVIRUS RESISTANCE 1* also showed that it confers resistance to potexviruses (Yamaji et al., 2012). *JAX1*-mediated resistance was identified in *A. thaliana* as a dominant gene, with some accessions possessing a stop codon at the beginning of the coding sequence, resulting in translational termination (Yamaji et al., 2012). These examples reveal that natural variations in a gene can affect its defensive actions against viruses. Furthermore, similar findings to what we observe in Col-0 and C24 *AGO2* have been reported for many NLRs. NLRs are responsible for recognizing cognate *Avr* effector proteins and confer resistance to pathogens (Eitas and Dangl, 2010). Natural variation in an *A. thaliana* NLR, RPS4 (*Resistance to Pseudomonas syringae* 4) from Col-0 and other accessions revealed that two amino acid changes are responsible for the loss of resistance to *P. syringae* (Narusaka et al., 2017).

Similar to the examples cited above, natural variation in some RNA silencing gene have also been reported to affect the outcome of plant-virus interaction. Closest to our study is the allelic variation of *Ty-1* and *Ty-3*, which are alleles of the same gene. Both encode a γ-class RdRp that confer resistance to tomato yellow leaf curl virus (TYLCV, a begomovirus) in tomatoes (Verlaan et al., 2013). It was reported that variation of four amino acids in the catalytic domain of *ty-1* is responsible for loss of its antiviral function and thus renders plants bearing this allele susceptible to TYLCV (Caro et al., 2015). This finding is similar to what we observed in the Col-0 and C24 *AGO2* alleles. In addition to the antiviral roles of dominant defense genes mentioned above, natural recessive resistance genes have been used to control viral pathogens have also been reported. An example is the eukaryotic initiation factors elF4E, elF4G and their isoforms (collectively called eIF4 factors) that play critical roles in the replication and translation of viral genomes and in assisting viral movement in plants (Machado et al., 2017; Truniger and Aranda, 2009). Mutations or variations in *eIF4* may be acquired in specific genes involved in cellular mechanism which are required for the infection cycle of the virus (Schmitt-Keichinger, 2019). Natural variation in the *eIF4E* gene has been identified and shown to be responsible for

resistance to potyvirus infection. Indeed, susceptibility to potato virus y (PVY) and lettuce mosaic virus (LMV), were restored in resistant cultivars by transiently expressing elF4E from a susceptible cultivar (Nicaise et al., 2003; Ruffel et al., 2002).

A major finding of the first project (Chapter 2) is the trade-off that exists between defense and reproduction in plants. Our results indicate that the C24 *AGO2* gene confers susceptibility to viruses but offers a reproductive advantage in the absence of virus infections. The trade-off between defense and growth/reproduction is abounds in the literature (Karasov et al., 2017). A recent study reported that the hypersusceptible *N. benthamiana* strain used in laboratory possesses a mutation in *RDR1*, a gene involved in RNA silencing, causing the plant to be more susceptible to viral infection even though *rdr1* plants exhibit early vigor and increased seed size (Bally et al., 2015). The above is an example of the delicate balance between evolutionary fitness and protection. In addition, the C24 *AGO2* is as functional as the Col-0 *AGO2* for defense against *P. syringae* (Brosseau et al., 2019). This shows that bacteria and viruses have induced independent selective pressures on the *AGO2* gene (Brosseau et al., 2019). The fact that the C24-like *AGO2* is prevalent in nature (Brosseau et al. 2019, Figure 2.1) shows that its role in the absence of viruses might be beneficial for plant fitness in the wild. In conclusion, genetic variation of genes involved in antiviral defense have been identified and used to generate virus resistant plants. These variations could also be relevant for other life history traits. Future work is needed to characterize natural variations that might be present in other *AGO* genes such as AGO4, which is essential for defense against geminiviruses and some RNA viruses, to see whether increased resistance to virus may be mediated by using genetic diversity in others AGO genes. The possibility of incorporating the antiviral variants of these genes into crops should also be explored. As shown in our study, some alleles are not effective for virus resistance but are important for other evolutionary arms-race relationships and fitness factors.

4.2 Inter-specific differences in Plant AGO2-The future of transgenic viral tolerant plants.

Plant viruses are a major yield-reducing factor for horticultural and field crops (Abiri et al., 2016). It has been reported that potyviruses and geminiviruses are responsible for the greatest number of

losses caused by plant viruses (Hohn and Vazquez, 2011; Ye et al., 2015), although disease caused by other groups of viruses are quickly becoming a serious concern (Varma et al., 2002). Potyviruses affect the yield of crops such as potato, sugarcane, papaya, vegetables and cereals (Ivanov et al., 2014; Jain et al., 1998; Revers and García, 2015) and the management of these viral diseases is of utmost importance. A strategy that has been used to generate resistant plants is the incorporation of an antiviral gene into susceptible plants to confer viral resistance. Prior to the work reported here, transgenic plants, which possess an increased tolerance to abiotic and biotic stresses have been engineered and commercialized (Lau et al., 2014) (Table 4.2). The engineering of intrinsic plant genes, related in the RNA silencing pathway, for virus resistance is the avenue that was explored and reported here. The discussion of previous methods used in the generation of virus-resistant transgenic plants will render the findings of our study as novel and ideal for developing virus-resistant plants.

Some plant genes that induce resistance to viruses (*R*-genes) have been employed to generate transgenic virus resistant plants (Table 4.1; Varma, 2001). This includes the *N* gene from tobacco which was introduced into tomatoes to confer protection against TMV and ToMV, two viruses that cause severe infections in tomatoes (Dinesh-Kumar et al., 2000). In addition, the Rx_1 and Rx_2 genes from the wild species of potato, *Solanum tuberosum ssp. andigena* and *Solanum acaule* respectively encode an extreme resistance to PVX, leading to little or no virus accumulation at the site of infection and no viral movement, thereby conferring resistance to the said virus. These genes have been introduced into other *S. tuberosum* species as well as some *Nicotiana* species to confer resistance to PVX (Bendahmane et al., 2000, 1999). Plantibodies, an antibody-based resistance has also been used to fortify plants against viral infections of viruses such as PVX, TBSV and TMV. Resistance conferred on plants by plantibody results from expressing virus-specific antibody fragments in transgenic plants (Gargouri-Bouzid et al., 2006; Liao et al., 2006; Matić et al., 2019). In addition, the introduction of genes conferring resistance to insects, into crops has also been studied (Xiao and Wu, 2019). Bt (*Bacillus thuringiensis)* endotoxin genes have been introduced into rice, tomato, maize, tobacco, potato, cotton and other crops and had been demonstrated to confer some viral resistance to such plants as it reduces some viruliferous insect populations (excluding aphids), thereby, decreasing the spread of some viral diseases. Lastly, aphids are a major crop pest that are responsible for significant annual loss of yield by inflicting

damage through the direct effects of feeding and by vectoring debilitating plant viruses (Wang et al., 2014). Therefore, as a strategy to manage this pest, the sequence specific nature of RNA silencing is being used to produce transgenic plants expressing dsRNAs designed to target genes of aphids (Sun et al., 2019). The successes recorded through these methods make it clear that the pursuit of crop plant engineering which confers viral resistance is essential and worthy of continued exploration. It is however important to note that there are risks associated with these methods. For instance, the use of plantibody as well as the generation of Bt crops involves the introduction of genes of animal and bacteria origins into plants. This has generated a level of push back against genetically modified (GM) crops by growers and consumers who are opposed to cross kingdom gene transfer (Lucht, 2015). A highpoint of the results in chapter 3 is that the genes employed in the study are of plant origin.

Another concept that has been reported is the coat protein-mediated resistance. This is a form of pathogen-derived resistance, where sequences of the coat protein (CP) of the virus of interest is engineered to be expressed in the plant (Table 4.1). The degree of protection ranges from a delay in symptoms, to absence of virus accumulation and disease manifestation. It has been established as an effective means of protection against viral infection and the prevention of crop loss (Prins et al., 2008). CP genes have been shown to confer partial or complete resistance as was observed for TSV in tobacco, *CMV* in cucumber, and *PVY* in potato plants (Prins et al., 2008). In *Cucurbita pepo* (squash), some of the significant virus diseases were caused by CMV and potyviruses such as zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV) (Lindo et al., 2017). Using CP-mediated resistance, expression cassettes, which consist of CP gene sequences of ZYMV, WMV, and CMV were incorporated into squash plants. Multi-virus resistant transgenic squash plants were developed. This resulted in the emergence of multi-virus resistant squash plants which were the first virus resistant transgenic plants commercially sold in the United States (Lindo et al., 2017). Soon after this, transgenic papaya that are resistant to papaya ringspot virus (PRSV), were also generated via CP-mediated resistance (Dennis Gonsalves, 2004; Ferreira and Pitz, 2002; Tecson Mendoza et al., 2008), and are equally approved for human consumption. The CP-mediated resistance is a very promising method for the generation of virus resistant plants. However, one of the initial concerns for this method was that it conferred a narrow resistance spectrum (Lindo et

al., 2017). To address this concern, studies have reported the relevance of CP-mediated resistance to RNA silencing. It was reported that tobacco plants transgenic for a non-translatable *Tobacco etch virus* (TEV) CP gene sequence (only expressed RNA), were immune to TEV infection. The transgene produced dsRNA in *planta* by efficiently base pairing with itself, and then used to degrade transgene transcripts as well as TEV genomic RNA, leading to resistance. With this finding, constructs containing virus CP gene sequences in inverted repeat (IR) orientation have been used to generate transgenes (Lindo et al., 2017). Majority of plant lines transformed with these IR of virus sequences showed virus resistance (Varsha Wesley et al., 2001). One of the advantages of the IR strategy is that the transcription of IRs of virus sequences are short, therefore, transgenes that confer resistance to multiple viruses have been designed. Using this IR approach, by assembling ~150 bp of sequence from four different tomato-infecting tospoviruses, simultaneous resistance has been engineered to all four viruses (Bucher et al., 2006). Therefore, genes related in the RNA silencing pathway such as *AGO* genes should be utilized for generating virus resistant plants.

4.3 How knowledge of AGO biology can enrich future transgenic approaches.

In the work presented in chapter 3, the possibility of introducing an *AGO2* gene from a model plant species to render a crop (tomato) resistant to a virus was explored. Previous work done in our lab also investigated the introduction of intra-species *AGO2* gene within species. The Col-0 *AGO2* gene was transferred to C24 plants (a PVX susceptible ecotype) and the resultant C24 plants were found to be resistant to PVX (Brosseau et al., 2019, Figure 5.6). Therefore, the introduction of a more effective *AGO* into a plant species is a viable method for the generation of transgenic virus resistant plants and thus, could be further explored. Another salient discovery in the work reported in chapter 2, was that a variant of the *AGO2* gene was discovered to confer a reproductive advantage. In the presence of viruses, the Col-0-like *AGO2* effectively restricted viral symptoms and plants possessing this gene were more reproductive than the C24-like *AGO2* plants (Figure 2.5). In the transgenic plants, the introduction of AtAGO2 (Col-0 AGO2) does not have an obvious phenotypic impact on plant fitness. With this, we can speculate that the introduction of *AtAGO2* (Col-0 AGO2) into crops may either promote yield or have no negative effect on yield in the presence of virus infections. This is an important finding because an increase in yield is essential to sustainable agriculture. However, future work is needed to explore the fitness of transgenic

AtAGO2 tomato plants as well as the impact of having multiple copies of *AtAGO2* in plants. Plants possessing multiple copies of AtAGO2 may be able to overcome the VSR activities of multiple viruses as viruses possess VSRs that effectively counteract the RNA silencing mechanism. In conclusion, chapter 3 sheds light on how genes involved in the RNA silencing pathways can be used to develop transgenic virus resistant plants. Further work such as the impact of the *AtAGO2* transgene on yield and other physiological traits should be explored. Future studies to combine several of the approaches described above to develop transgenic plants with broad resistance to viral infections should also be investigated. Such multi-virus resistant plants are needed during synergistic virus infections.

Over the years, many of the examples cited above have been shown to confer long-term resistance regardless of the evolution of the viruses they effect defense against. However, because AGO proteins are a target of several VSRs, further studies would be required to determine if the strategy employed for the generation of virus resistant plants in chapter 3 is a long-term solution. This is because plant viruses have been reported to evolve rapidly with both RNA and DNA viruses possessing a high rate of mutation (Sanjuán et al., 2010). In addition, VSRs are very efficient and have been reported to have evolved independently as they possess remarkable sequence diversity and structure across and within kingdoms (Csorba et al., 2015). Thus, the rapid evolution of plant viruses and the diversity of VSRs imply diversity in viruses and VSR mechanistic activities, which could cause our strategy to be inefficient. Therefore, to address this possibility, future work to incorporate *AtAGO2* into transgenic *N. benthamiana* plants possessing the Rx_1 and Rx_2 genes, could be developed. It is expected that this combination will work in synergy to further increase plant resistance to virus infections based on findings from work presented here and other studies.

Bioinformatic resources are worthy of exploration even though they were not used in work presented in chapters 2 and 3. It is my recommendation that future work should employ this very important research tool to enhance data generation and analysis. It is my expectation that bioinformatics will provide a high-resolution data analysis tool for the precise insertion of Col-0 AtAGO2 into crops to achieve higher level of virus resistance by analyzing sequences from multiple genes in whole plant genomes. Analysis such as metagenomics and transcriptomics data

obtained from wild plant populations could shed light on other genes in the RNA silencing pathway that possess natural variations for defense against viruses. A finding that will shed more understanding on understanding the implications of these variations. It is equally recommended that gene editing technology could be brought to bear on this important field. To broaden the study of the underlying differences between Col-0 AGO2, C24 AGO2 and NbAGO2, gene editing tool such as CRISPR/Cas9 could be used as a strategy to supplement the traditional plant transformation method used in our study to generate these virus resistant plants. Some of the advantages of using gene-editing instead of the conventional breeding techniques is that less time might be spent to produce the desired transgenic plants and introduction of the transgenic gene will be precise. Generating virus-resistant AGO transgenic plants is essential and should be explored further. Since plants already possess endogenous AGO proteins, the modification of some of these endogenous AGOs will confer a tremendous advantage on the plants.

Table 4.1- Summary of transgenic interference with factors affected during plant-virus interactions.

Transgene	**Transgenic Interference**
Virus derived • **Coat protein** • **Replicase protein** • **Movement protein** • **Viral protease** • **Helper protein** • **Seed transmission factor** • **Non-coding region** • **Antisense RNA/DNA** • **Satellite** • **Defective interfering RNAs**	 • Transmission, Uncoating, Assembly • Replication • Invasion • Protein processing • Delays symptoms development • Seed transmission • Competition for viral replicase • Replication, translation, assembly • Replication • Replication
Plant-derived	

• **PAP/PAPII** • **AtAGO2** • **Host '*R*' genes**	• Multiplication • Yet to be tested. Proposed: multiplication • Multiplication
Others • **Plantibodies** • **Yeast RNase**	 • Replication, protein processing, assembly • Cleaving of dsRNA

4.4 References

Bendahmane, A., Kanyuka, K., Baulcombe, D.C., 1999. The Rx Gene from Potato Controls Separate Virus Resistance and Cell Death Responses. Plant Cell 11, 781–791.

Bendahmane, A., Querci, M., Kanyuka, K., Baulcombe, D.C., 2000. Agrobacterium transient expression system as a tool for the isolation of disease resistance genes: application to the Rx2 locus in potato. Plant J. 21, 73–81. https://doi.org/10.1046/j.1365-313x.2000.00654.x

Bucher, E., Lohuis, D., van Poppel, P.M.J.A., Geerts-Dimitriadou, C., Goldbach, R., Prins, M., 2006. Multiple virus resistance at a high frequency using a single transgene construct. J. Gen. Virol. 87, 3697–3701. https://doi.org/10.1099/vir.0.82276-0

Carbonell, A., Carrington, J.C., 2015. Antiviral roles of plant ARGONAUTES. Curr. Opin. Plant Biol. 27, 111–117. https://doi.org/10.1016/j.pbi.2015.06.013

Caro, M., Verlaan, M.G., Julián, O., Finkers, R., Wolters, A.-M.A., Hutton, S.F., Scott, J.W., Kormelink, R., Visser, R.G.F., Díez, M.J., Pérez-de-Castro, A., Bai, Y., 2015. Assessing the genetic variation of Ty-1 and Ty-3 alleles conferring resistance to tomato yellow leaf curl virus in a broad tomato germplasm. Mol. Breed. 35, 132. https://doi.org/10.1007/s11032-015-0329-y

Cosson, P., Schurdi-Levraud, V., Le, Q.H., Sicard, O., Caballero, M., Roux, F., Le Gall, O., Candresse, T., Revers, F., 2012. The RTM resistance to potyviruses in Arabidopsis thaliana: natural variation of the RTM genes and evidence for the implication of additional genes. PLoS One 7, e39169. https://doi.org/10.1371/journal.pone.0039169

Cosson, P., Sofer, L., Le, Q.H., Léger, V., Schurdi-Levraud, V., Whitham, S.A., Yamamoto, M.L., Gopalan, S., Le Gall, O., Candresse, T., Carrington, J.C., Revers, F., 2010. RTM3, which controls long-distance movement of potyviruses, is a member of a new plant gene family encoding a meprin and TRAF homology domain-containing protein. Plant Physiol. 154, 222–32. https://doi.org/10.1104/pp.110.155754

Csorba, T., Kontra, L., Burgyán, J., 2015. Viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. Virology. https://doi.org/10.1016/j.virol.2015.02.028

Decroocq, V., Salvador, B., Sicard, O., Glasa, M., Cosson, P., Svanella-Dumas, L., Revers, F., García, J.A., Candresse, T., 2009. The Determinant of Potyvirus Ability to Overcome the RTM Resistance of *Arabidopsis thaliana* Maps to the N-Terminal Region of the Coat Protein. Mol. Plant-Microbe Interact. 22, 1302–1311. https://doi.org/10.1094/MPMI-22-10-1302

Dennis Gonsalves, 2004. Transgenic Papaya in Hawaii and Beyond.

Dinesh-Kumar, S.P., Tham, W.-H., Baker, B.J., Gajiwala, P., Pasion, R., Whalen, M.C., Stall, R.E., Staskawicz, B.J., 2000. Structure-function analysis of the tobacco mosaic virus resistance gene N. Proc. Natl. Acad. Sci. 97, 14789–14794. https://doi.org/10.1073/pnas.97.26.14789

Eitas, T.K., Dangl, J.L., 2010. NB-LRR proteins: Pairs, pieces, perception, partners, and pathways. Curr. Opin. Plant Biol. https://doi.org/10.1016/j.pbi.2010.04.007

Ferreira, S.A., Pitz, K.Y., 2002. Virus Coat Protein Transgenic Papaya Provides Practical Control of Papaya ringspot virus in Hawaii.

Gargouri-Bouzid, R., Jaoua, L., Rouis, S., Saídi, M.N., Bouaziz, D., Ellouz, R., 2006. PVY-Resistant Transgenic Potato Plants Expressing an Anti-NIa Protein scFv Antibody. Mol. Biotechnol. 33, 133–140. https://doi.org/10.1385/MB:33:2:133

Hohn, T., Vazquez, F., 2011. RNA silencing pathways of plants: Silencing and its suppression by plant DNA viruses ☆. BBA - Gene Regul. Mech. 1809, 588–600. https://doi.org/10.1016/j.bbagrm.2011.06.002

Ivanov, K.I., Eskelin, K., Lõ, A., Mä Kinen Correspondence, K., Mä, K., 2014. Molecular and

cellular mechanisms underlying potyvirus infection. J. Gen. Virol. 95, 1415–1429. https://doi.org/10.1099/vir.0.064220-0

Jain, R.K., Pappu, H.R., Pappu, S.S., Varma, A., Ram, R.D., 1998. Molecular characterisation of papaya ringspot potyvirus isolates from India. Ann. Appl. Biol. 132, 413–425. https://doi.org/10.1111/j.1744-7348.1998.tb05218.x

Lau, W., Fischbach, M.A., Osbourn, A., Sattely, E.S., 2014. Key applications of plant metabolic engineering. PLoS Biol. 12, e1001879. https://doi.org/10.1371/journal.pbio.1001879

Liao, Y.-C., Pingli, H., Senzhao, C., Yao, M.-J., Zhang, J.-B., Liu, J.-L., Lia0, Y.-C., 2006. Biotechnology and Genetic Engineering Reviews Plantibodies: A Novel Strategy to Create Pathogen-Resistant Plants 13 Plantibodies: A Novel Strategy to Create Pathogen-Resistant Plants. Biotechnol Genet Eng Rev. 23, 253–71. https://doi.org/10.1080/02648725.2006.10648087

Lucht, J.M., 2015. Public Acceptance of Plant Biotechnology and GM Crops. Viruses 7, 4254–81. https://doi.org/10.3390/v7082819

Machado, J.P.B., Calil, I.P., Santos, A.A., Fontes, E.P.B., 2017. Translational control in plant antiviral immunity. Genet. Mol. Biol. 40, 292–304. https://doi.org/10.1590/1678-4685-GMB-2016-0092

Mahajan, S.K., Chisholm, S.T., Whitham, S.A., Carrington, J.C., 1998. Identification and characterization of a locus (RTM1) that restricts long-distance movement of tobacco etch virus in Arabidopsis thaliana. Plant J. 14, 177–186. https://doi.org/10.1046/j.1365-313X.1998.00105.x

Matić, S., Noris, E., Contin, R., Marian, D., Thompson, J.R., 2019. Engineering partial resistance to cucumber mosaic virus in tobacco using intrabodies specific for the viral polymerase. Phytochemistry 162, 99–108. https://doi.org/10.1016/J.PHYTOCHEM.2019.03.006

Narusaka, M., Iuchi, S., Narusaka, Y., 2017. Analyses of natural variation indicates that the absence of RPS4/RRS1 and amino acid change in RPS4 cause loss of their functions and resistance to pathogens. Plant Signal. Behav. 12. https://doi.org/10.1080/15592324.2017.1293218

Nicaise, V., German-Retana, S., Sanjuán, R., Dubrana, M.-P., Mazier, M., Maisonneuve, B., Candresse, T., Caranta, C., LeGall, O., 2003. The Eukaryotic Translation Initiation Factor 4E Controls Lettuce Susceptibility to the Potyvirus Lettuce mosaic virus . Plant Physiol. 132, 1272–1282. https://doi.org/10.1104/pp.102.017855

Rambod Abiri, al, Valdiani, A., Maziah, M., Azmi Shaharuddin, N., Sahebi, M., Norhana Balia Yusof, Z., Atabaki, N., Talei, D., Valdiani alireza, A., 2016. A Critical Review of the Concept of Transgenic Plants: Insights into Pharmaceutical Biotechnology and Molecular Farming. Curr. Issues Mol. Biol. 18, 21–42. https://doi.org/10.21775/cimb.018.021

Revers, F., García, J.A., 2015. Molecular Biology of Potyviruses. Adv. Virus Res. 92, 101–199. https://doi.org/10.1016/BS.AIVIR.2014.11.006

Ruffel, S., Dussault, M.H., Palloix, A., Moury, B., Bendahmane, A., Robaglia, C., Caranta, C., 2002. A natural recessive resistance gene against potato virus Y in pepper corresponds to the eukaryotic initiation factor 4E (eIF4E). Plant J. 32, 1067–1075. https://doi.org/10.1046/j.1365-313X.2002.01499.x

Sanjuán, R., Nebot, M.R., Chirico, N., Louis, M., Belshaw, R., Sanjua, R., Mansky, L.M., 2010. Viral Mutation Rates Viral Mutation Rates □. J. Virol. 84, 9733–9748. https://doi.org/10.1128/JVI.00694-10

Schmitt-Keichinger, C., 2019. Manipulating cellular factors to combat viruses: A case study from the plant eukaryotic translation initiation factors eIF4. Front. Microbiol. https://doi.org/10.3389/fmicb.2019.00017

Sun, Y., Sparks, C., Jones, H., Riley, M., Francis, F., Du, W., Xia, L., 2019. Silencing an essential gene involved in infestation and digestion in grain aphid through plant-mediated interference generates aphid-resistant wheat plants. Plant Biotechnol. J. 17, 852–854. https://doi.org/10.1111/pbi.13067

Tecson Mendoza, E.M., C. Laurena, A., Botella, J.R., 2008. Recent advances in the development of transgenic papaya technology. Biotechnol. Annu. Rev. 14, 423–462. https://doi.org/10.1016/S1387-2656(08)00019-7

Truniger, V., Aranda, M.A., 2009. Recessive Resistance to Plant Viruses. Adv. Virus Res. 75,

119–231. https://doi.org/10.1016/S0065-3527(09)07504-6

Varma, A. and M.N., 2001. Durable host-plant resistance, a desirable trait for integrated disease management.

Varsha Wesley, S., Helliwell, C.A., Smith, N.A., Wang, M., Rouse, D.T., Liu, Q., Gooding, P.S., Singh, S.P., Abbott, D., Stoutjesdijk, P.A., Robinson, S.P., Gleave, A.P., Green, A.G., Waterhouse, P.M., 2001. Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J. 27, 581–590. https://doi.org/10.1046/j.1365-313X.2001.01105.x

Verlaan, M.G., Hutton, S.F., Ibrahem, R.M., Kormelink, R., Visser, R.G.F., Scott, J.W., Edwards, J.D., Bai, Y., 2013. The Tomato Yellow Leaf Curl Virus Resistance Genes Ty-1 and Ty-3 Are Allelic and Code for DFDGD-Class RNA-Dependent RNA Polymerases. PLoS Genet. 9.

Wang, D., Liu, Q., Jones, H.D., Bruce, T., Xia, L., 2014. Comparative transcriptomic analyses revealed divergences of two agriculturally important aphid species. BMC Genomics 15. https://doi.org/10.1186/1471-2164-15-1023

Xiao, Y., Wu, K., 2019. Recent progress on the interaction between insects and Bacillus thuringiensis crops. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2018.0316

Ye, J., Yang, J., Sun, Y., Zhao, P., Gao, S., Jung, C., Qu, J., Fang, R., Chua, N.H., 2015. Geminivirus Activates ASYMMETRIC LEAVES 2 to Accelerate Cytoplasmic DCP2-Mediated mRNA Turnover and Weakens RNA Silencing in Arabidopsis. PLoS Pathog. 11.

APPENDIX - SUPPLEMENTAL MANUSCRIPT

Natural variation in the *Arabidopsis AGO2* gene controls susceptibility to PVX

In this article, we first show that AGO proteins from *A. thaliana* and *N. benthamiana* show differing activities against PVX, suggesting that inter-specific differences in AGO2 may contribute to differing outcomes of PVX infection in these species. Furthermore, we tested whether intra-specific differences in AGO2 might affect plant-virus interactions by taking advantage of the natural genetic variation of wild *A. thaliana* accessions. We show the *AGO2* gene presents a high level of polymorphism and that, unlike the commonly used *A. thaliana* accession Col-0, some naturally occurring accessions that were analyzed were susceptible to PVX. This susceptibility is determined by two polymorphisms found in the N-terminus of the AGO2 protein, suggesting that natural variation in AGO2 may be important for determining plant-virus interaction outcomes.

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5. Natural variation in the *Arabidopsis AGO2* gene is associated with susceptibility to PVX

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5.1 Abstract

RNA silencing functions as an anti-viral defence in plants through the action of DICER-like (DCL) and ARGONAUTE (AGO) proteins. Despite the importance of this mechanism, there are few known examples of natural genetic variants in genes encoding RNA silencing components that affect virus susceptibility. The AGO2 protein is important for antiviral defense against multiple viruses and has been shown to be a major limiting factor to infection by potato virus X (PVX) of *Arabidopsis thaliana* but not *Nicotiana benthamiana*. We show that the AGO2 proteins from these two plants have differential activity against PVX, suggesting that variation in AGO2 is important in plant-virus interactions. Consistent with this, we find that the *Arabidopsis thaliana AGO2* gene shows a high incidence of polymorphisms between accessions, with evidence for selection. AGO2 protein variants can be assigned to two groups, based on similarity to the proteins found in the Col-0 or C24 accessions, respectively. This involves an amino acid change associated with small deletions in the N-termini of C24-like AGO2 proteins. Inoculation of a large number of *Arabidopsis* accessions shows a very strong correlation between Col-0 and C24 *AGO2* alleles with resistance and susceptibility to PVX, respectively. These observations were validated using genetic and transgenic complementation analysis, which showed that C24 AGO2 is specifically affected in its antiviral activity, without interfering with other AGO2-associated functions such as anti-bacterial resistance or DNA methylation. Our results suggest that plant-virus interactions have

influenced natural variation in RNA silencing components and that the latter may be an avenue for improving resistance to viruses in crop plants.

5.2 Introduction

RNA silencing is a conserved gene regulatory mechanism that is also employ by plants to counteract virus infection (Carbonell and Carrington, 2015). Virus double-stranded RNA (dsRNAs), produced during the replication of RNA viruses, is recognized and cleaved into small-interfering RNAs (siRNAs) by dicer-like (DCL) proteins. These siRNAs are then incorporated into an RNA-silencing complex (RISC) which contains Argonaute (AGO) endoribonucelase proteins. These programmed complexes subsequently target any single-stranded (ssRNA) with sufficient complementarity for cleavage or translation inhibition (Omarov et al., 2016). To counteract RNA silencing, plant viruses encode viral suppressors of RNA silencing (VSR), which have been shown to interfere with multiple different steps of this mechanism (Pumplin and Voinnet, 2013). The P25 protein, also known as TGB1, of potato virus X (PVX) is implicated in viral movement, in the formation of viral replication complexes (VRC), and functions as a VSR by inducing a destabilization of AGO proteins (Bayne et al., 2005; Chiu et al., 2010; Tilsner et al., 2012; Voinnet et al., 1999).

Among the ten AGO proteins encoded by *Arabidopsis thaliana*, AGO2 has been identified most frequently as having antiviral function and this, against multiple different viruses (Carbonell and Carrington, 2015). Indeed, AGO2 appears to be a major determinant of the inability of PVX to systemically infect *Arabidopsis* accession Col-0 (Jaubert et al., 2011). An antiviral role for AGO2 is conserved in *Nicotiana benthamiana* (Fátyol et al., 2016; Ludman et al., 2017; Odokonyero et al., 2015; Scholthof et al., 2011). However, despite the presence of a functional AGO2, *N. benthamiana* is susceptible to a wide range of viruses, including PVX (Bally et al., 2015). Although multiple studies have elucidated how AGO proteins carry out their basic biochemical functions (Fátyol et al., 2016; Poulsen et al., 2013), less is known about why certain AGO proteins function more or less effectively against viruses. Likewise, natural variation in AGO proteins

within and between species has not been explored for its potential roles in plant-virus interactions and co-evolution.

Here, we have shown that AGO proteins from *A. thaliana* and *N. benthamiana* show differing activities against PVX, suggesting that inter-specific differences in AGO2 may contribute to differing outcomes of PVX infection in these species. Furthermore, we tested whether intra-specific differences in AGO2 might affect plant-virus interactions by taking advantage of the natural genetic variation of wild *Arabidopsis* accessions. We show the *AGO2* gene presents a high level of polymorphism and that, unlike the commonly used *Arabidopsis* accession Col-0, 27 out of 63 accessions analyzed are susceptible to PVX. This susceptibility is determined by two polymorphisms found in the N-terminus of the AGO2 protein, suggesting that natural variation in AGO2 may be important for determining plant-virus interaction outcomes.

5.3 Results

5.3.1 AGO2 proteins from different genera display specific antiviral activity

To determine if differences in PVX susceptibility between *N. benthamiana* and *A. thaliana* might be determined in part by AGO2, we transiently expressed the two proteins with PVX-GFP. Consistent with our previous results(Brosseau and Moffett, 2015), transient expression of AtAGO2 in *N. benthamiana* resulted in a lower PVX-derived GFP accumulation (Figures 5.1a and b). However, NbAGO2 had much less effect on PVX accumulation, as determined visually and by immunoblotting (Fig. 5.1a, 5.1b). Despite being expressed under the same strong promoter, the two AGO2 proteins did not accumulate at similar levels in this experiment (Figure 5.1b, middle panel). Previous studies have shown that the Potexvirus VSR, P25, has the potential to compromise stability or accumulation of different AGO proteins (Brosseau et al., 2016; Brosseau and Moffett, 2015; Chiu et al., 2010). To monitor whether the presence of P25 affects NbAGO2 antiviral activity, we used a mutant version of PVX, PVX-GFPΔTGB, which lacks P25. In contrast to WT PVX, both AGO2 proteins significantly reduced the accumulation of virus-derived GFP from PVX-GFPΔTGB (Figure 5.1c, 5.1d). We also noticed that At and Nb AGO2 accumulated at similar

levels when co-expressed with PVXΔTGB (Figure 5.1d) suggesting that P25 may affect the two different AGO2 proteins differently. Consistent with this, expression of AGO2 proteins with P25 alone reduced NbAGO2 accumulation, but not AtAGO2 (Figure 5.1e). Taken together, these results suggest that P25 VSR activity is at least partially responsible for the differential efficiency in targeting PVX observed between these two AGO2 proteins.

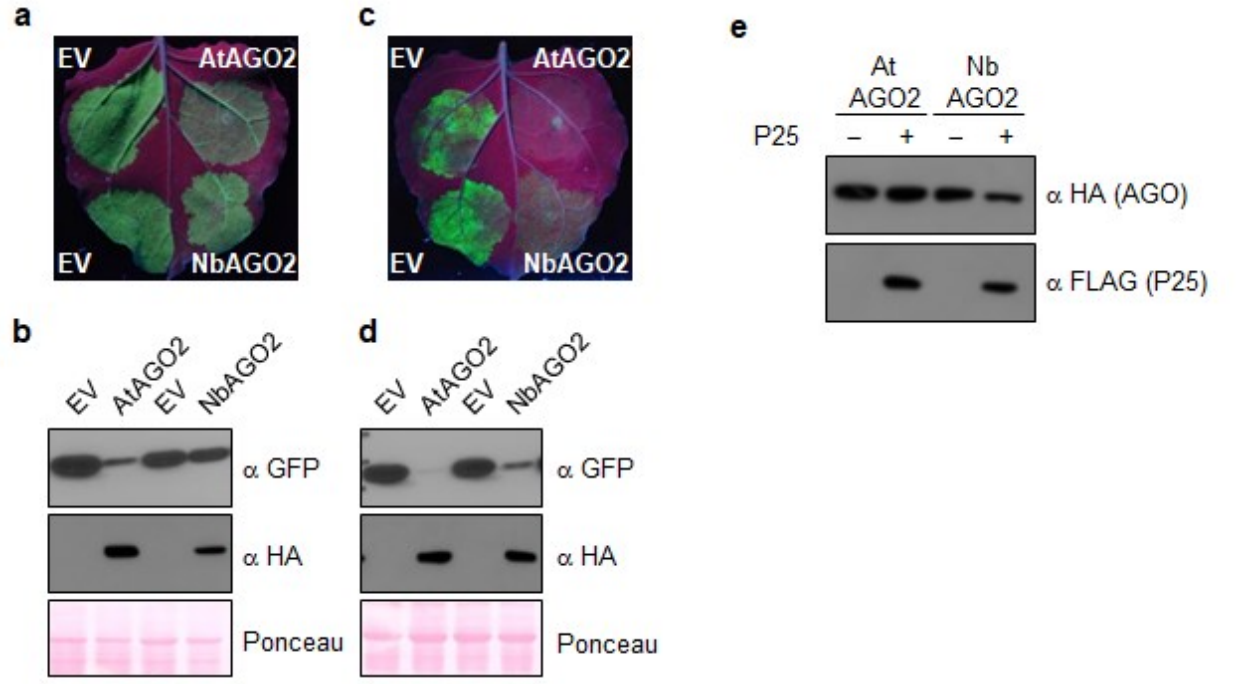

Figure 5.1. AtAGO2, but not NbAGO2, shows anti-viral activity against PVX. a and **c**, *N. benthamiana* leaves were agroinfiltrated with PVX-GFP WT **a** or ΔTGB **c** along with 35S:HA-AtAGO2, 35S:HA-NbAGO2 or empty vector (EV). Leaves were photographed under UV illumination at 4 days post infiltration (dpi). **b** and **d**, Total protein extracts were prepared from *N. benthamiana* leaves agroinfiltrated as in **a** and **c** at 4 dpi and subjected to SDS-PAGE, followed by anti-GFP immunoblotting (top panel). HA-tagged AGO proteins were immunoprecipitated from the same extracts and subjected to anti-HA immunoblotting (middle panel). Ponceau staining (bottom panel) of the same extracts is shown to demonstrate equal loading. **e**, HA-tagged AGO proteins were co-expressed by agroinfiltration in *N. benthamiana* leaves with either FLAG-tagged P25 or with empty vector (EV). Total proteins were extracted and subjected to anti-FLAG

immunoblotting (bottom panel). HA-tagged AGO proteins were immunoprecipitated and subjected to anti-HA immunoblotting (top panel).

5.3.2 The *Arabidopsis AGO2* gene displays a high degree of polymorphism

The NbAGO2 and AtAGO2 proteins are only 50% identical (Nakasugi et al., 2013), which may explain their being differentially affected by PVX P25 but makes it difficult to identify specific residues important for anti-PVX activity. To evaluate whether the *AGO2* gene shows differences within a species, we analyzed the coding sequences of *AGO1* (At1g48410) and *AGO2* (At1g31280) from 80 *Arabidopsis* accessions, representing eight Eurasian geographic regions(Cao et al., 2011), as obtained from the 1001 genomes project (The 1001 Genomes Consortium, 2016) and by sequencing for some accessions (Supplementary Table 1). Upon analysis, we observed that multiple alleles possessed short indels (compared to the reference Col-0 allele), mainly located in the 5' end of the *AGO2* coding sequence resulting in extensive variation in the resulting protein lengths, ranging from 993 to 1014 amino acids. We also found a high level of single-nucleotide polymorphisms (SNP) throughout the *AGO2* coding sequence (Figure 5.2a and Table S5.1). Synonymous SNPs are six times more frequent in the *AGO2* coding region than that of *AGO1*, while non-synonymous SNPs are more than fifty times more frequent (Table S5.1 and 2; Figure 5.2a), suggesting that *AGO2* has been subjected to selection pressure.

Selective pressures on AGO2 sequences were evaluated by analyzing the ratio of nonsynonymous (*dN*) to synonymous substitution rates per site (d*S*). Site-by-site analysis indicated that residue 33 (Col-0 allele) showed the strongest signal of having undergone positive selection pressure (Figure 5.2b). In the 80 accessions analyzed, only two different amino acids are found at position 33, namely an aspartic acid or a glycine (Table S5.1). The Col-0 accession encodes an aspartic acid at position 33 whereas many other accessions, including the commonly used C24 accession, encodes

a glycine at the equivalent position (Figure 5.2c). Interestingly, the presence of a glycine at residue 33 is almost always correlated with deletions, ranging from 2 to 13 amino acids, in the region of the protein N-terminal to residue 33, which encodes a number of GR repeats (Figure 5.2b), as is seen in the C24 accession (Figure 5.2c). Given this strong correlation, we refer to *AGO2* alleles encoding 33D as Col-0-like and those encoding 33G plus a GR deletion as C24-like, although C24 *AGO2* possesses 2 additional SNPs, A110D and T131S, and an insertion of a valine at residue 134.

Although multiple *AGO2* alleles showed additional indels and SNPs, none of these individual differences were present at high prevalence. In the set of 80 Eurasian *Arabidopsis* accessions Col-0-like and C24-like alleles are present at a frequency of 50% and 43.75%, respectively (Figure S5.2d) and both alleles are found in all eight sub-populations (Figure S5.1a). Only three variants with a full GR motif plus G33 and only two variants with a GR deletion plus D33 were identified, which we refer to collectively as rare *AGO2* alleles (Figure 5.2d and Figure S5.1a). Interestingly, the presence of an aspartic acid at the equivalent of residue 33 of AGO2 appears to be the exception in the *Brassicaceae* family, with other species investigated encoding a glycine at the equivalent residue (Figure S5.1b).

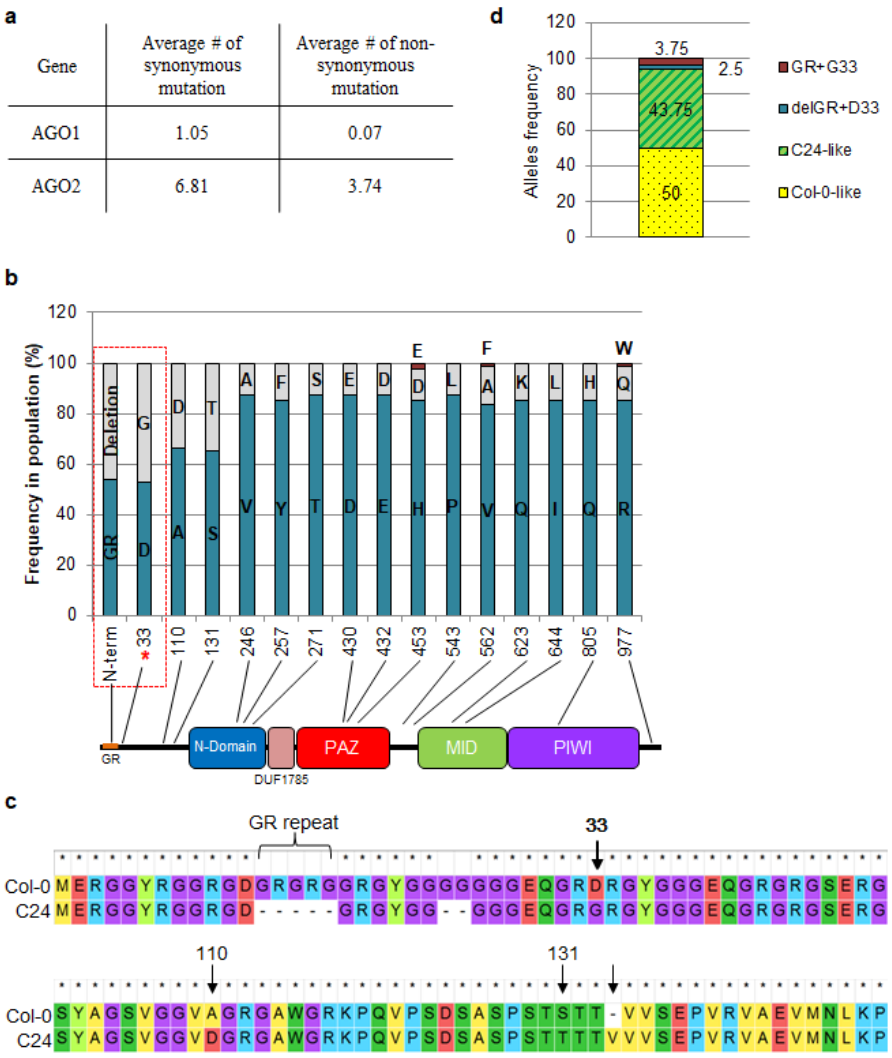

Figure 5.2. Residue 33 of *Arabidopsis AGO2* has undergone positive selection in natural populations. a, Variability observed in *AGO1* and *AGO2* sequences in 80 Eurasian *Arabidopsis* accessions in comparison to the Col-0 accession. **b**, Chart showing AGO2 residues having a high frequency of non-synonymous polymorphisms in the 80 accessions. A red dotted rectangle identifies polymorphisms frequently co-occurring in AGO2. An asterisk indicates an amino acid variant found to be subjected to positive selection pressure with a posterior probability of >95%, supported by fixed-effects likelihood (FEL) (Kosakovsky Pond and Frost, 2005) with a *p* value < 0.1 using DataMonkey.org website. Different domains of AGO2 are schematically represented by rectangles (not to scale) under the chart. Blue: N-terminal, red: PAZ, green: MID and purple: PIWI. **c**, Alignment of the N-terminal region of AGO2 protein of Col-0 and C24 AGO2 alleles. **d**, Graph

representing allele frequencies in 80 different Eurasian accessions based on polymorphisms found in GR motif and residue 33 of AGO2 N-terminal region.

5.3.3 C24-like *AGO2* alleles are strongly associated with systemic infection by PVX in *Arabidopsis*.

To test whether sequence variation observed in AGO2 might influence antiviral activity, we inoculated 63 accessions from the eight different populations with PVX. Susceptibility or resistance was scored based on the detection of PVX CP in upper non-inoculated tissues by immune blotting. We observed that, unlike Col-0, multiple accessions were susceptible to PVX and that resistance or susceptibility did not correlate with geographic origin (Figure 5.3a; Figures S5.2 and S5.3; Table S5.1). However, we found that 27 out of 30 tested accessions possessing a Col-0-like *AGO2* were resistant to PVX (Figure 5.3; Table S5.1). Conversely, 24 out of 31 tested accessions having either a C24-like or rare *AGO2* allele were susceptible to PVX (Figure 5.3; Table S5.1). The *JAX1* gene has been shown previously to confer broad-spectrum resistance to potexviruses and its presence varies between *Arabidopsis* accessions (Yamaji et al., 2012). We thus determined the *JAX1* status (+/-) in all accessions tested (Figure 5.3; Table S5.1). After doing so, we found that all resistant accessions with a C24-like *AGO2* allele, except Copac-1, also possessed a functional *JAX1* gene (Figure 5.3). Together, these results indicate that variability in *AGO2* sequence is strongly associated with susceptibility to PVX in *Arabidopsis*.

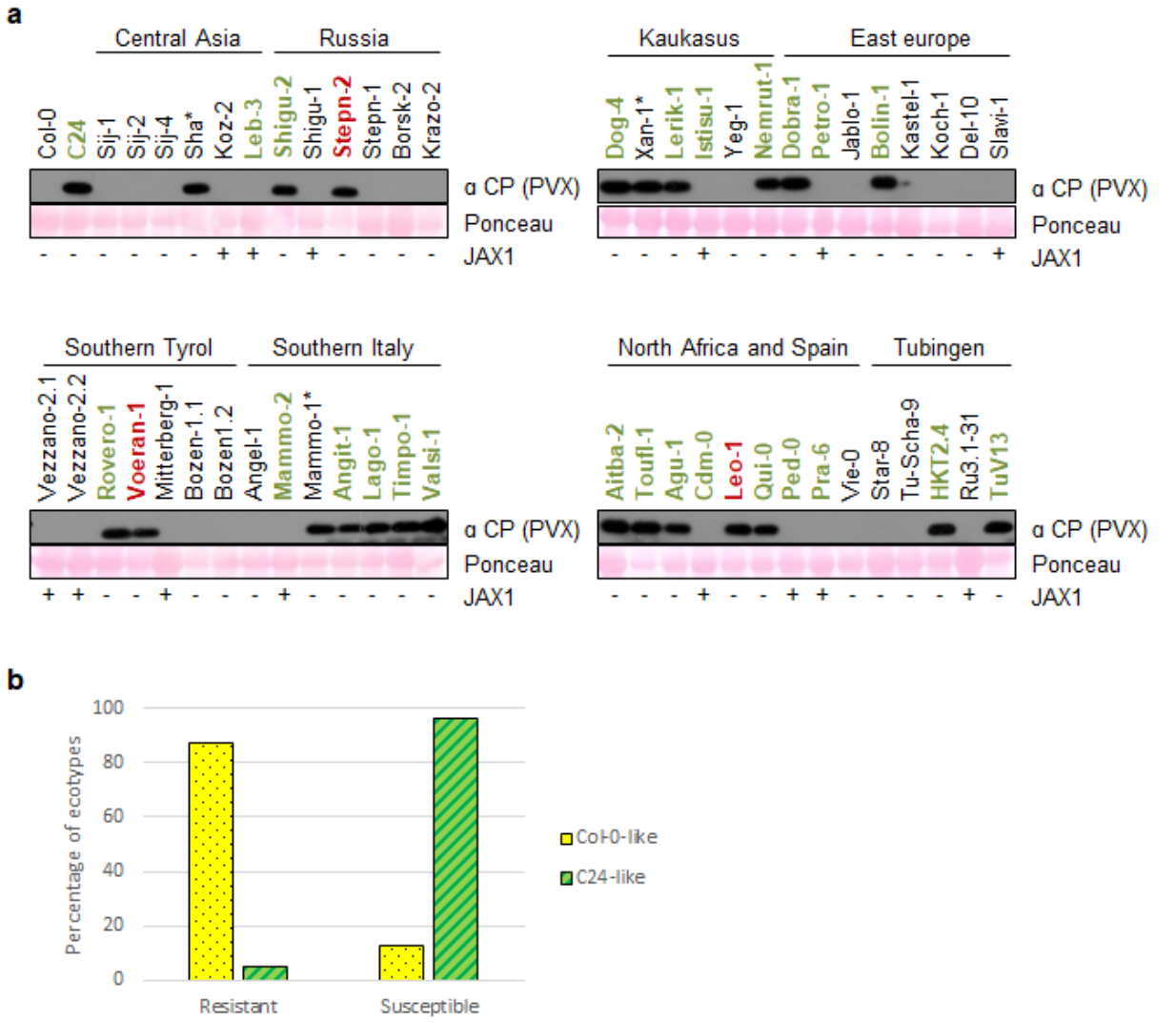

Figure 5.3. Natural variation in the N-terminus of AGO2 correlates with susceptibility to PVX. a, Different *Arabidopsis* accessions were inoculated with PVX, as indicated, categorized by geographic region of origin. At 21 dpi, total protein extracts were prepared from upper non-inoculated leaves and subjected to SDS-PAGE followed by anti-PVX CP immune blotting. Ponceau staining (bottom panel) of the same extracts is shown to demonstrate equal loading. Accession names are colored according to their AGO2 allele: Col-0-like are black, C24-like are bold green and rare alleles are bold red. Asterisks indicate accessions that do not fit the expected correlation. Accessions were also genotyped *in silico* for the presence or absence (+/-) of a functional *JAX1* allele. Note that less protein sample was loaded into wells for C24, Stepn-2 and Bolin-1 due to the strong accumulation of PVX in these accessions. **b**, Compilation of results

obtained for all JAX (-) accessions tested as in **a**. The Pearson's r-coefficient is 0.9958207 and falls within a 95% confidence interval.

5.3.4 Validation of the effect of different AGO2 alleles and susceptibility to PVX in reciprocal inbred lines

Although we observed a strong association between *AGO2* alleles and PVX susceptibility, we cannot rule out that polymorphisms in other genes might contribute to the observed phenotypes due to the high level of genetic diversity between the accessions tested (Cao et al., 2011). We thus took advantage of previously described reciprocal introgression lines (RILs). These lines were derived by crossing Col-0 and C24, followed by iterative backcrossing to the parental genotypes and selfing, resulting in lines with a majority of one parental genotype, with relatively small genomic regions derived from the other (Törjék et al., 2008). From this collection, we selected RILs wherein the *AGO2* alleles were exchanged between accessions, as well as control lines wherein genomic regions adjacent to, but not including *AGO2*, were exchanged (Figure 5.4a and Table S5.3). RILs used in this study are depicted in Figure 4a and the origin of the *AGO2* allele was verified for each line by PCR (Figure S5.4a). We then assessed these lines for susceptibility to PVX. In agreement with our previous report (Jaubert et al., 2011), no PVX was detected in the upper non-inoculated tissues of Col-0, but was detected in more than half of the *ago2-1* mutant plants tested (Figure 5.4b and Figure S5.4b). Similarly, PVX was not detected in upper non-inoculated tissues of control RILs N35, N62 and N66, which have both Col-0 background and the Col-0 *AGO2* allele. However, PVX was detected in upper non-inoculated leaves of more than half of infected plants from N37 and N55 lines wherein the C24 *AGO2* allele has been introgressed into a Col-0 background (Figure 5.4b). Conversely, introgression of the Col-0-like *AGO2* allele into the C24 background (RIL M39) resulted in resistance to PVX similar to Col-0 plants (Figure 5.4b and Figure S5.4b). A Pearson's r-coefficient test has shown a statistically significant positive correlation between the *AGO2* allele of the plant and its accumulation of PVX in upper non-inoculated leaves (Figure 5.4c).

The use of RILs between Col-0 and C24 accessions allows us to test the involvement of AGO2 alleles in virus resistance in the absence of potentially confounding effects of transgenes. At the same time however, several genes in this region of chromosome I display polymorphisms including *AGO3*. Phylogenetically, *AGO3* is the closest homologue to *AGO2* however, except against BaMV, *AGO3* has not been found to be required for virus resistance (Alazem et al., 2017; Brosseau et al., 2016; Brosseau and Moffett, 2015; Garcia-Ruiz et al., 2015a; Jaubert et al., 2011), but rather appears to play a role in DNA methylation (Zhang et al., 2016). Moreover, although C24 *AGO3* possesses some polymorphisms, *in silico* analysis revealed that these polymorphisms are also found in resistant accessions (Table S5.3) precluding its implication in PVX susceptibility phenotype.

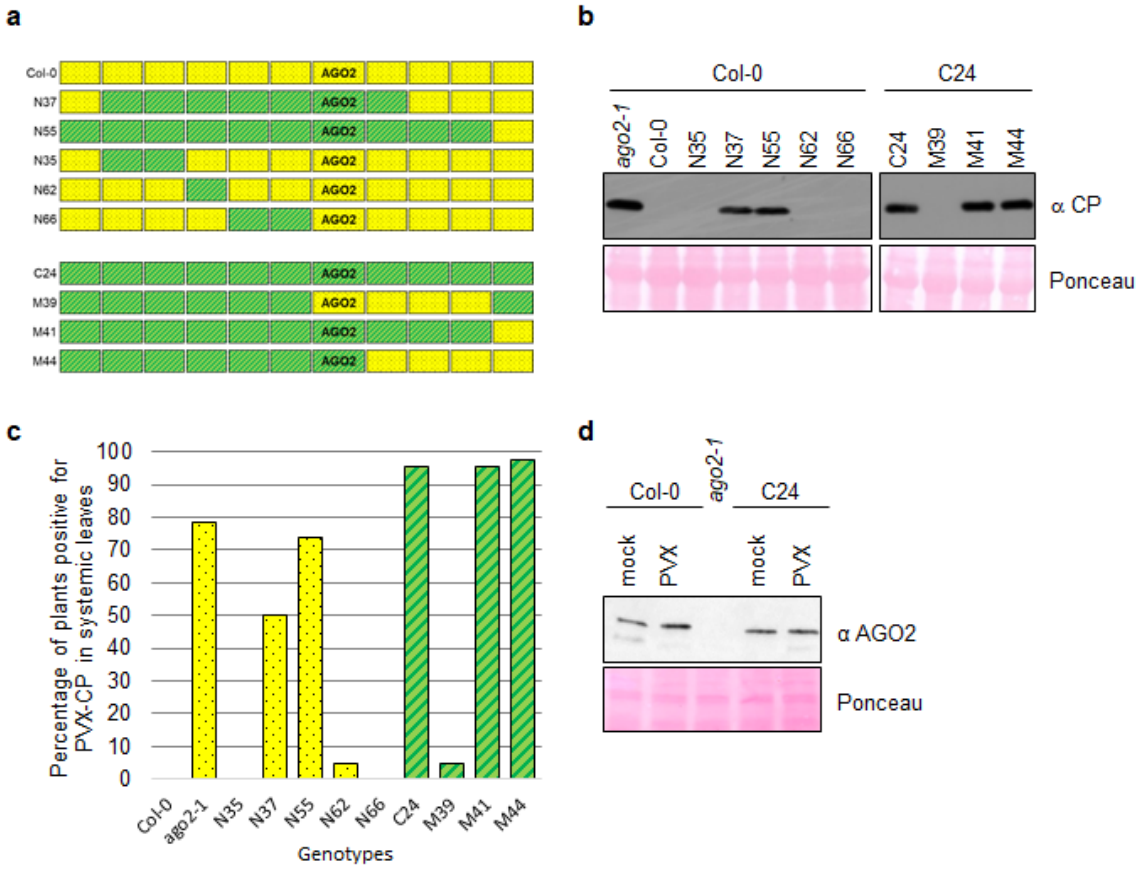

Figure 5.4. Exchange of AGO2 alleles between Col-0 and C24 changes susceptibility to PVX. **a**, Schematic representation of the region of chromosome I between nt 174607 and nt 22286233

in recombinant intogression lines (RILs) from a cross between Col-0 and C24 (Törjék et *al*., 2008). N lines have a Col-0 background (yellow) throughout the genome except for small regions of chromosome I substituted with the corresponding region from C24 (green). M lines have a C24 background (green) with Col-0 substitutions (yellow). **b**, RILs, as depicted in **a**, were inoculated with PVX. At 21 dpi, total protein extracts from upper non-inoculated leaves were prepared and subjected to SDS-PAGE followed by anti-PVX CP immune blotting (top panel). Ponceau staining (bottom panel) of the same extracts is shown to demonstrate equal loading. Representative results from 42 replicates are shown. **c**, Compilation of results obtained for all replicates tested as in **b** (related to Supplementary Fig. 2b). The Pearson's r-coefficient is 0.6140612 and falls within a 95% confidence interval. **d**, Col-0, WT or *ago2-1*, and C24 plants were inoculated with PVX. At 21 dpi, total protein extracts from upper non-inoculated leaves were prepared and subjected to SDS-PAGE followed by anti-AGO2 (Agrisera antibody) immune blotting (top panel). Ponceau staining (bottom panel) of the same extracts is shown to demonstrate equal loading.

Exogenous application of the phytohormone salicylic acid has been shown to compromise PVX accumulation in *N. benthamiana* (Naylor et al., 1998) and *ICS1,* a key salicylic acid (SA) biosynthesis gene, is significantly up-regulated in C24 compared to Col-0 (Yang et al., 2015). However, quantification of SA in different RILs showed that introgression of the Col-0 genomic region containing the *AGO2* gene into the C24 background, or the opposite exchange, does not significantly change SA accumulation in these lines, relative to the parental genotypes (Supplementary Fig. 4c) thus precluding a role for SA in the observed phenotypes. Moreover, the differential susceptibility cannot be attributed to differential AGO2 expression as both accessions display similar AGO2 protein accumulation in upper non-inoculated leaves upon inoculation with PVX (Fig. 4d). These results further validate the involvement of AGO2 polymorphism as a major determinant of resistance of to PVX in *Arabidopsis*.

5.3.5 C24 *AGO2* is not a null allele

In *Arabidopsis*, AGO2 has also been implicated in antibacterial defense responses, presumably through its binding to endogenous miRNAs, as well as in the methylation of some DNA loci (Pontier et al., 2012; Zhang et al., 2011). Aside from the differences found in the N-terminus outlined above, the C24 AGO2 protein does not differ from Col-0 in any of the well-characterized functional AGO domains. To determine whether C24 AGO2 is still efficient in non-virus-related functions, we inoculated RILs with virulent *Pseudomonas syringae* pv. tomato (Pto). In Col-0 background lines in which C24 *AGO2* has been introgressed, namely N37 and N55, Pto grew at titres similar to WT and at significantly lower titers compared to *ago2-1* (Col-0 background) mutant plants (Figure 5.5a). Likewise, introgression of Col-0 *AGO2* into C24 had no significant effect on bacterial growth compared to WT C24 (Figure 5.5a). This suggests that C24 *AGO2* polymorphisms do not compromise the function of this protein in response to bacterial infection. Furthermore, we observed that Pst infection induces *AGO2* expression at similar level in both accessions (Figure 5.5b), consistent with previous reports (Zhang et al., 2011).

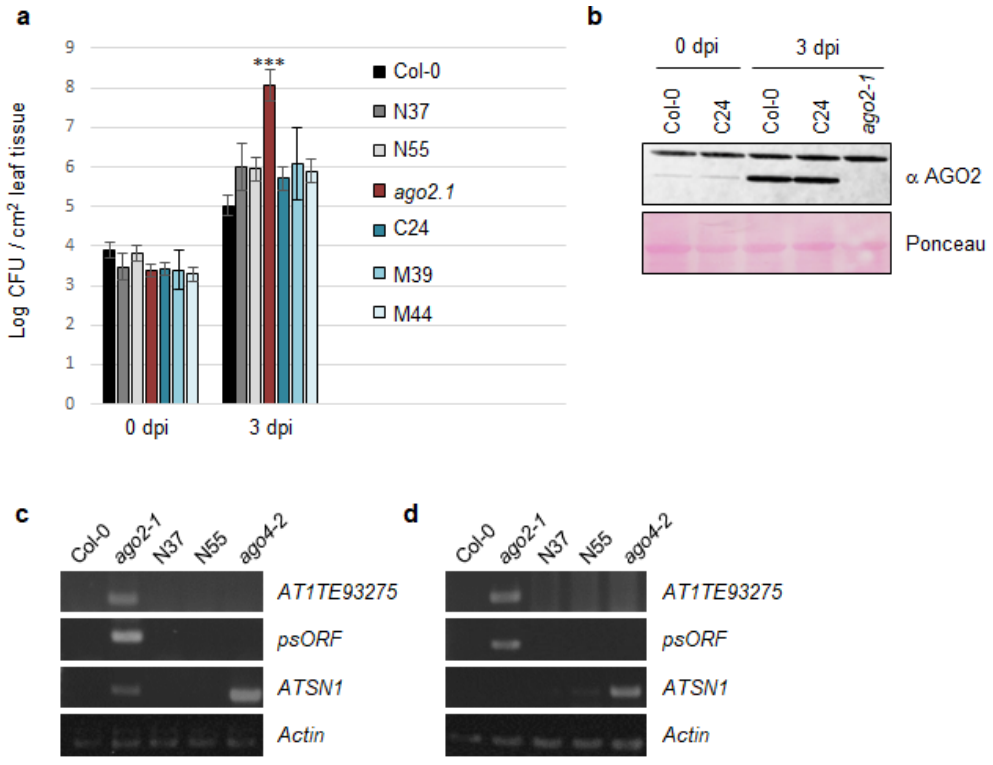

Figure 5.5. C24 AGO2 retains anti-bacterial and methylation-related functions. a, The indicated RILs with Col-0 and C24 backgrounds were infected with virulent Pst. Bacteria were counted at 0 and 3 dpi. Error bars indicate SEM from three biological replicates. Asterisks indicates statistically significant differences (student t test) at a P-value < 0.005. **b**, Total protein extracts were prepared from *Arabidopsis* leaves inoculated as in (A) at 0 and 3 dpi and subjected to SDS-PAGE, followed by anti-AGO2 (Abiocode) immunoblotting (top panel). Ponceau staining (bottom panel) of the same extracts is shown to demonstrate equal loading. **c**, Genomic DNA from the indicated genotypes was isolated and subjected to digestion with McrBC and subjected to PCR with primers to the indicated loci. **d**, RNA from the indicated genotypes was isolated and subjected to RT-PCR using primers to the indicated loci.

A previous study has reported that DNA methylation and gene silencing of the psORF and AT1TE93275 transposable elements are compromised in *ago2* mutant plants (Pontier et al., 2012). To examine whether C24 AGO2 is still functional in this respect, DNA methylation status and expression of AT1TE93275 and psORF were verified in two Col-0 lines containing the C24 *AGO2* allele. Genomic DNA from Col-0, *ago2-1*, N37, N55 and *ago4-2* plants was isolated and digested with the methylation dependent restriction enzyme McrBC. As shown in Figure 5.5c, the *ago2-1* mutant shows amplification of psORF and AT1TE93275 after McrBC digestion, whereas the N37 and N55 lines do not, while all lines show similar amplification of the actin gene (Fig. 5c). As expected, a similar test showed greater amplification of ATSN1, a signature locus for AGO4-dependent methylation, in the *ago4* mutant (Figure 5.5c). At the same time, RT-PCR analysis showed that psORF and AT1TE93275 could be amplified only from RNA extracted from the *ago2-1* mutant and ATSN1 showed significant amplification only in the *ago4-2* mutant (Figure 5.5d). These results are highly consistent with previous reports (Pontier et al., 2012) and indicate that both the Col-0 and the C24 AGO2 proteins are functional for AGO2-dependent methylation and its associated repression of a transposable element. Taken together, these results suggest that a complete GR motif and D33 of the Col-0 AGO2 protein are required for optimal antiviral defense but appear to be dispensable for regulating endogenous transcripts and methylation-related functions.

5.3.6 C24 AGO2 shows decreased antiviral activity against PVX compared to Col-0 AGO2.

Manual alignment of NbAGO2 and AtAGO2 proteins shows that NbAGO2 encodes a glycine at the position equivalent to G33 of C24-AGO2 (Figure S5.5). Because NbAGO2 was found to be efficiently antiviral only against a VSR-deficient version of PVX (Figure 5.1), we verified whether it behaved similarly to C24 AGO2 in a transient overexpression assay. Indeed, in transient assays, C24 AGO2 is as efficient as Col-0 AGO2 at restricting PVX-GFPΔTGB but is somewhat less efficient against WT PVX-GFP (Figure S5.6a, b ,c, d). This effect was also seen with other Col-0-like and C24-like AGO2 alleles, Yeg-1 and Bolin-1, respectively (Figure S5.6 a ,b, c, d). The difference between 35S-driven transient expression C24 vs. Col-0 AGO2 is not as dramatic as the difference seen with NbAGO2. However, expressing the different AGO2 variants under the native NbAGO2 promoter enhance the differential activity of these proteins against WT PVX (Figure 5.6a, b) as well as against PlAMV-GFP, another Potexvirus (Figure S5.7 a, b).

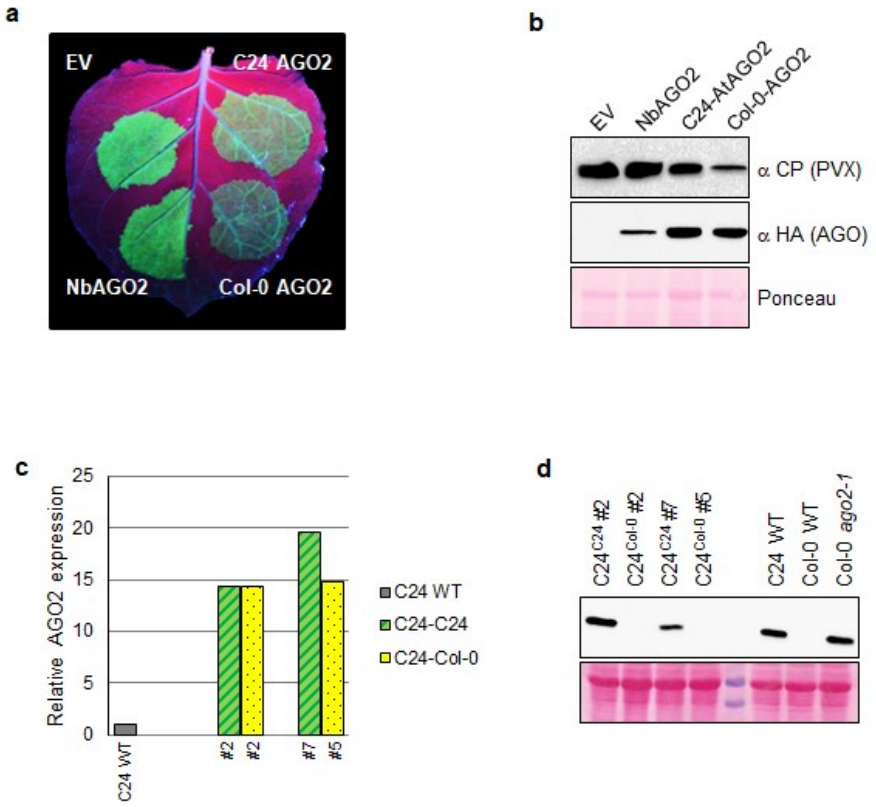

Figure 5.6. Polymorphisms found in C24 *AGO2* affect its antiviral activity in *Arabidopsis*.

a, *N. benthamiana* leaves were agroinfiltrated with PVX-GFP, along with pNbAGO2:HA-NbAGO2, pNbAGO2:HA-AtCol-0-AGO2, pNbAGO2:HA-AtC24-AGO2 or empty vector (EV). Leaves were photographed under UV illumination at 4 dpi. **b**, Total protein extracts were prepared from *N. benthamiana* leaves agroinfiltrated as in **a** at 4 dpi and subjected to SDS-PAGE, followed by anti-PVX CP (top panel) or anti-HA (middle panel) immune blotting. Ponceau staining (bottom panel) of the same extracts is shown to demonstrate equal loading. **c** and **d**, WT C24, as well as different lines (#2, 5, 2 and 7) of transgenic plants expressing the indicated transgene, were inoculated with PVX. At 21 dpi, upper non-inoculated leaves were harvested for qRT-PCR analysis **c** and immune blotting **d**. **c**, Total RNA was extracted from upper non-inoculated leaves and subjected to qRT-PCR analysis to determine relative expression levels of total *AGO2*. Relative expression of *AGO2* in transgenic lines, $C24^{C24}$ and $C24^{Col\text{-}0}$, is normalized to the relative expression of *AGO2* in WT C24. **d**, Total protein extracts from upper non-inoculated leaves were prepared and subjected to SDS-PAGE followed by anti-PVX CP immune blotting (top panel). Ponceau staining (bottom panel) of the same extracts is shown to demonstrate equal loading.

To verify whether this differential antiviral activity between Col-0-AGO2 and C24-AGO2 is biologically relevant in whole plants, we created C24 transgenic lines expressing Col-0-AGO2 or C24-AGO2 under the control of the 35S promoter. AGO2 transgene expression was then monitored by qRT-PCR as, despite being under the control of a strong promoter, both proteins were undetectable by immune blotting analysis (data not shown). Lines with similar *AGO2* expression levels ($C24^{C24}$#2 and $C24^{Col\text{-}0}$#2) were analyzed by immune blotting to verify their susceptibility to PVX (Fig. 6c, 6d). At 21 dpi, PVX was not detected in upper non-inoculated leaves of $C24^{Col\text{-}0}$ lines showed whereas $C24^{C24}$#2 showed similar accumulation of PVX compared to that of C24 WT. In addition, the $C24^{C24}$#7 line, expressing almost 20X more AGO2 than in C24 WT, remains susceptible to PVX as determined by immunoblotting (Fig. 6c, 6d). These results suggest that polymorphisms found in C24-AGO2 compromised its antiviral activity, and that AGO2 expression levels are not responsible for these differences. Furthermore, combined with the RILs analysis, it also validates our conclusion that AGO2 polymorphisms are a major genetic determinant in resistance/susceptibility phenotypes observed in different *Arabidopsis* accessions.

5.4 Discussion

5.4.1 Susceptibility to PVX is common in *Arabidopsis thaliana*

Although the widely used *Arabidopsis* Col-0 accession is resistant to systemic PVX infection, we find that a large number of natural accessions are susceptible (Figure 5.3). There are many potential barriers to compatible interactions between plants and viruses. However, our findings are consistent with studies showing that *Arabidopsis* encodes the necessary components to support Potexvirus- replication (Hashimoto et al., 2016; Jaubert et al., 2011; Keima et al., 2017) and that abrogation of the RNA silencing machinery is sufficient to render Col-0 an effective host for PVX (Andika et al., 2015; Brosseau and Moffett, 2015; Jaubert et al., 2011; Yamaji et al., 2012). Combined with our findings that differential effectiveness of versions of AGO2 from different species and accessions, this suggests that natural variation in RNA silencing components may play important roles in host range determination and in ecological dynamics of plant-virus interactions.

5.4.2 High prevalence of polymorphisms in the *AGO2* coding sequence

Surveys of *A. thaliana* accessions have revealed extensive natural allelic variation, comprising SNPs as well as indels (Koornneef et al., 2004; Nordborg et al., 2005; Zhang et al., 2008). These naturally occurring variations are often associated with resistance to various biotic factors, particularly at disease resistant (R) gene loci encoding NLR proteins (Bakker et al., 2006.; Borevitz et al., 2007; Clark et al., 2007). The *AGO2* coding region has 50 times more non-synonymous SNPs than the *AGO1* coding region (Figure 5.2a; Table S5.1 and S5.2). This suggests that AGO2 has been subjected to strong selective pressure for diversification. Such selection would be consistent with a study in *Drosophila* showing that genes related to antiviral RNAi evolve at a

significantly faster rate than paralogous genes implicated in housekeeping RNAi functions (miRNA pathway) (Obbard et al., 2006). Likewise, a broader study suggests that viruses have driven close to 30% of all adaptive amino acid changes in mammals, making them dominant drivers of protein adaptation (Enard et al., 2016). Importantly, in our analysis, we found no polymorphisms in residues predicted to be important for sRNA loading and maturation, RNA binding, AGO hook or catalytic functions (Fátyol et al., 2016). Although variation has been observed throughout the coding sequence of AGO2, the only residue found to be under positive selection was in the N-terminus, that is to say, outside of conserved catalytic domains (Figure 5.2), similar to what was observed in mammal RNAi-related proteins (Obbard et al., 2006). Consistent with the retention of core AGO function (Figure 5.5) of C24 AGO2, this suggests that polymorphisms may have been selected for due to interactions with viruses. Indeed, all plant viruses are thought to encode at least one VSR, some of which interact directly with RNAi proteins, including AGOs, and thus host antiviral RNAi components must rapidly adapt to win the molecular arms race against pathogens (Mukherjee et al., 2013; Obbard et al., 2006). At the same time, VSRs must evolve to overcome the RNA silencing machineries of their hosts, which may explain why PVX P25 affects NbAGO2, having evolved with *Solanaceous* hosts, but not AtAGO2 (Figure 5.1).

Both Col-0-like and C24-like alleles have remained prevalent in Eurasian populations at near equal frequencies (Figure 5.2). Balancing selection on alleles conferring differing degrees of disease resistance is thought to occur because of trade-offs between fitness and defense under differing environmental conditions (Mauricio, 1998; Todesco et al., 2010). Indeed, Col-0-like AGO2 variants may confer greater virus resistance but have a negative effect on plant fitness. Alternatively, it is possible that C24-like *AGO2* alleles are more functional against other naturally-occurring viruses in *A. thaliana* or that it confers some other fitness advantage unrelated to its role in antiviral defense. Such conservation of a non-functional allele of a gene involved in antiviral silencing has been described for the *RDR1* gene in *N. benthamiana* (Bally et al., 2015) wherein a functional antiviral allele negatively impacts early vigour in *N. benthamiana* (Bally et al., 2015). Likewise, the frequent occurrence of non-functional *JAX1* alleles (Yamaji et al., 2012) (Figure 5.3, Table S5.1) may be due to a fitness penalty for this type of resistance to potexviruses. However, AGO2 differs from these examples in that the C24-like variants retain most of their inherent

activities (Figure 5.5). Interestingly, it has been reported that *AGO2* has undergone positive selection in tomato during the domestication process (Koenig et al., 2013), although what function has been selected for is unclear.

The most frequent polymorphisms in the *AGO2* coding sequence, ΔGR and D33G, are both found in a region of the protein identified as Block 43, (Figure 5.2b, Figure 5.3) (Rodríguez-Leal et al., 2016). This motif is present in different copy numbers in different AGO proteins from animal and plant species and, although well conserved, its function is unknown (Rodríguez-Leal et al., 2016). It is of notable that in nearly all cases these two polymorphisms are either both absent or both present (Figure 5.2), suggesting that their pairing may have some functional significance. Indeed, many C24-like variants have different GR deletions, suggesting that they may have arisen independently, possibly as to compensate for a loss of function due to having an aspartate at residue 33. Such a recurrent selection for alleles non-functional for disease resistance would be similar to selection at certain NLR loci, such as RPM1, where multiple loss of function alleles have been selected for over evolutionary time (Rose et al., 2012; Stahl et al., 1999).

Among *Arabidopsis* AGO proteins, AGO1, 2, 3, 5 and 10 encode multiple B43 motifs (Rodríguez-Leal et al., 2016). In numerous animal systems and more recently in *Arabidopsis*, arginine residue within this block have been shown to be methylated by PMRT (Hu et al., 2019; Kirino et al., 2010, 2009; Musiyenko et al., 2012; Siomi et al., 2010; Vagin et al., 2009). In *Arabidopsis*, bacterial infection results in a reduction in expression of *PMRT5*, which in turn reduces arginine methylation of AGO2, leading to stabilization of the protein and miRNA loading(Hu et al., 2019). Consistent with this, we also observe greater accumulation of AGO2 during bacterial infection (Figure 5.5b) and, to a much lesser extent during PVX infection in Col-0 (Fig. 4d) but not C24. The D33G modification introduces an additional GR repeat in C24-like AGO2 proteins that may be more accessible for residual *PMRT5* activity. Thus, the ΔGR deletions result in potentially less arginines that could be modified. However, since both Col-0 and C24 appear to accumulate to similar levels (Figure 5.4, Figure 5.5, Figure 5.6), and because both AGO2 proteins have similar functions in antibacterial defense and DNA methylation (Figure 5.4), we deem it unlikely that these polymorphisms impair protein stability or sRNA loading. Alternatively, we suggest that the

differences may prevent the P25 VSR activity from inhibiting Col-0-AGO2, although this will require further study.

5.5 Conclusion

We demonstrate that natural variation in a core RNA silencing protein, between and within species, is a major factor determining susceptibility to a wild-type virus. These results have important implications for plant-virus coevolution and suggest that identification of natural variants in AGO2 in crop and related species may prove useful in developing crops with increased resistance to virus pathogens.

5.6 Methods

5.6.1 Plant material and growth conditions

Nicotiana benthamiana and *Arabidopsis thaliana* plants were grown in soil (BM6, Berger and Agromix, Fafard PLACE, respectively) in growth chambers with 16-h-light/8-h-dark and 12-h-light/8-h-dark photoperiod at 23°C and 21°C respectively. Col-0 (CS28168), C24 (CS28127) and *Arabidopsis* wild accessions (CS76427) were obtained from the ABRC stock center. The *ago2-1* mutant in Col-0 background has been described elsewhere(Takeda et al., 2008). RILs between Col-0 and C24 were kindly provided by R.C. Meyer and have been described previously(Törjék et al., 2008).

5.6.2 Plasmid construction and transient expression

The *NbAGO2* ORF was cloned using cDNA derived from TBSV infected *N. benthamiana* leaves using primers listed in Supplemental Table 3. For the generation of different *Arabidopsis* AGO2 expression clones, cDNA (Fig. 1) and gDNAs (Fig. 6, Supplementary Fig. 6 and Supplementary Fig. 7) of the appropriate accessions were used as templates for PCR amplification with primers listed in Supplemental Table 3. PCR products were purified and cloned into the pGEM-T easy vector (Promega) and subcloned into the XbaI and BamHI sites of pBIN61 vector containing an N-terminal FLAG epitope in frame with the XbaI site or in pBIN61 empty vector for HA-tagged NbAGO2. All other constructs have been previously described including PVX, PVX-GFP, PVX-GFPΔTGB and PlAMV-GFP binary constructs (Bhattacharjee et al., 2009; Lu et al., 2003; Peart et al., 2002a; Yamaji et al., 2012), as well as FLAG-P25 (Brosseau and Moffett, 2015) and pBIC-HA-AtAGO2 (Takeda et al., 2008). For the generation of an AGO2 expression vector under the control of the *NbAGO2* promotor, AGO2 upstream regulatory sequences (2068 pb) were amplified from genomic DNA with primers listed in Supplemental Table 2. PCR products were purified and cloned into the pGEM-T easy vector (Promega) and subcloned into the Acc65I and XbaI sites and BamHI sites of pBIN61 vector to replace the 35S promoter. *Agrobacterium*-mediated transient expression (agroinfiltration) assays in *N. benthamiana* were performed as previously described (Brosseau and Moffett, 2015).

5.6.3 Virus inoculation

Infections of three-week-old *Arabidopsis thaliana* plant were carried out by rub inoculation as previously described (Brosseau and Moffett, 2015). Briefly, saps were produced with PVX-infected *N. benthamiana* plant material. Infected material was ground in 0.1 M phosphate buffer, pH 7.0 (2 mL/g of infected tissue). Mock inoculations were performed with sap produced with uninfected *N. benthamiana* plants (2 mL/g of healthy tissues).

5.6.4 Protein extraction and analysis

Protein extraction and analysis were carried out as previously described(Brosseau and Moffett, 2015). Proteins were detected by immune blotting using anti-HA-horseradish peroxidase conjugated (HRP) antibodies (Sigma, 1:3,000 dilution), anti-FLAG-HRP antibodies (Sigma, 1:5,000 dilution), anti-GFP-HRP antibodies (Santa Cruz, 1:3,000 dilution), anti-PVX-CP rabbit polyclonal antibodies (Agdia, 1:3,000 dilution) and anti-AGO2 antibody (Agrisera, 1:5,000 dilution or Abiocode 1:7,500). Detection of the latter three primary antibodies was performed using donkey anti-IgG rabbit-HRP polyclonal antibodies (BioLegend, 1:10,000 dilution).

5.6.5 Gene expression and DNA methylation analysis

Total RNA was isolated with Trizol (Ambion) and subjected to RT-PCR using primers listed in Supplemental Table 2. Gene expression and McrBC analyses were performed as previously described(Pontier et al., 2012) with minor modifications. 10 µg of genomic DNA were digested with 6u of McrBC (NEB) in a final volume of 50 µl for 3h at 37°C. The digested DNA was then analyzed by semi-quantitative PCR using primers listed in Supplementary Table 4.

RT-qPCR was performed as previously described (Brosseau et al., 2016; El Oirdi et al., 2011) using primers listed in Supplementary table 4.

5.6.6 Quantification of SA

Quantification of SA was performed by PhenoSwitch Bioscience Inc. (Sherbrooke, Canada). Briefly, salicylic acid was extracted from crushed tissues by the addition of 500µl of methanol containing 0.01 ng/µl of internal standard (D4-Salicylic acid). The samples were then incubated at 4°C for 30 minutes with end-over-end mixing and the insoluble material was cleared by centrifugation. The supernatant was diluted tenfold in water and the pH was adjusted to 7 by the addition of 50 mM ammonium acetate. A standard curve containing 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.91, 1.95 or 0.97 µg/ml SA was prepared in 50 µl H2O and processed the same way as the plant samples. For weak anion exchange solid phase extraction (WAX SPE) of salicylic acid

and internal standard, the protocol was as follows: phase conditioning with acetonitrile, wash with 50 mM ammonium acetate, sample loading, wash with 50 mM ammonium acetate, elution with 5% ammonium hydroxide in water. The eluate was dried down by speed vac, reconstituted in 50µl of water containing 0.2% formic acid and 10 mM ammonium formate and processed by LC-MS/MS.

5.7 References

Adams, M.J., Antoniw, J.F., Fauquet, C.M., 2005. Molecular criteria for genus and species discrimination within the family Potyviridae. Arch. Virol. 150, 459–479. https://doi.org/10.1007/s00705-004-0440-6

Adenot, X., Elmayan, T., Lauressergues, D., Boutet, S., Bouché, N., Gasciolli, V., Vaucheret, H., 2006. DRB4-Dependent TAS3 trans-Acting siRNAs Control Leaf Morphology through AGO7. Curr. Biol. 16, 927–932. https://doi.org/10.1016/j.cub.2006.03.035

Agorio, A., Vera, P., 2007. ARGONAUTE4 is required for resistance to Pseudomonas syringae in Arabidopsis. Plant Cell 19, 3778–90. https://doi.org/10.1105/tpc.107.054494

Akbergenov, R., Si-Ammour, A., Blevins, T., Amin, I., Kutter, C., Vanderschuren, H., Zhang, P., Gruissem, W., Meins, F., Hohn, T., Pooggin, M.M., 2006. Molecular characterization of geminivirus-derived small RNAs in different plant species. Nucleic Acids Res. 34, 462–471. https://doi.org/10.1093/nar/gkj447

Alazem, M., He, M.-H., Moffett, P., Lin, N.-S., 2017a. Abscisic Acid Induces Resistance against Bamboo Mosaic Virus through Argonaute2 and 3. Plant Physiol. 174, 339–355. https://doi.org/10.1104/pp.16.00015

Alazem, M., He, M.-H., Moffett, P., Lin, N.-S., 2017b. Abscisic Acid Induces Resistance against *Bamboo Mosaic Virus* through *Argonaute 2* and *3*. Plant Physiol. 174, 339–355. https://doi.org/10.1104/pp.16.00015

Anandalakshmi, R., Marathe, R., Ge, X., Herr, J.M., Mau, C., Mallory, A., Pruss, G., Bowman, L., Vance, V.B., 2000. A Calmodulin-Related Protein That Suppresses Posttranscriptional

Gene Silencing in Plants. Science (80-.). 290.

Andika, I.B., Maruyama, K., Sun, L., Kondo, H., Tamada, T., Suzuki, N., 2015. Differential contributions of plant Dicer-like proteins to antiviral defences against potato virus X in leaves and roots. Plant J. 81, 781–793. https://doi.org/10.1111/tpj.12770

Ange´lique Deleris, Javier Gallego-Bartolome, Jinsong Bao, Kristin D. Kasschau, James C. Carrington, O.V., 2006. Hierarchical Action and Inhibition of Plant Dicer-Like Proteins in Antiviral Defense. Science (80-.). 305, 1462–322. https://doi.org/10.1126/science.1128650

Axtell, M.J., 2013. Classification and Comparison of Small RNAs from Plants. Annu. Rev. Plant Biol. 64, 137–159. https://doi.org/10.1146/annurev-arplant-050312-120043

Azevedo, J., Garcia, D., Pontier, D., Ohnesorge, S., Yu, A., Garcia, S., Braun, L., Bergdoll, M., Hakimi, M.A., Lagrange, T., Voinnet, O., 2010. Argonaute quenching and global changes in Dicer homeostasis caused by a pathogen-encoded GW repeat protein. Genes Dev. 24, 904–915. https://doi.org/10.1101/gad.1908710

Bakker, E.G., Toomajian, C., Kreitman, M., Bergelson, J., n.d. A Genome-Wide Survey of R Gene Polymorphisms in Arabidopsis W. https://doi.org/10.1105/tpc.106.042614

Bally, J., Nakasugi, K., Jia, F., Jung, H., Ho, S.Y.W., Wong, M., Paul, C.M., Naim, F., Wood, C.C., Crowhurst, R.N., Hellens, R.P., Dale, J.L., Waterhouse, P.M., 2015. The extremophile Nicotiana benthamiana has traded viral defence for early vigour. Nat. Plants 1, 1–6. https://doi.org/10.1038/nplants.2015.165

Balogun, O.S., 2003. Patterns of disease manifestation in tomato seedlings singly or doubly infected with Potato X potexvirus and Tobacco Mosaic tobamovirus.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using **lme4**. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Baumberger, N., Baulcombe, D.C., 2005. Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. Proc. Natl. Acad. Sci. U. S. A. 102, 11928–33.

Bayne, E.H., Rakitina, D. V., Morozov, S.Y., Baulcombe, D.C., 2005. Cell-to-cell movement of Potato Potexvirus X is dependent on suppression of RNA silencing. Plant J. 44, 471–482. https://doi.org/10.1111/j.1365-313X.2005.02539.x

Bejerman, N., Mann, K.S., Dietzgen, R.G., 2016. Alfalfa dwarf cytorhabdovirus P protein is a local and systemic RNA silencing supressor which inhibits programmed RISC activity and prevents transitive amplification of RNA silencing. Virus Res. 224, 19–28. https://doi.org/10.1016/j.virusres.2016.08.008

Belgorodski, N.K.S.K.T., 2017. Package "rriskDistributions" Type Package Title Fitting Distributions to Given Data or Known Quantiles.

Bellaoui, M., Keddie, J.S., Gruissem, W., 2003. DCL is a plant-specific protein required for plastid ribosomal RNA processing and embryo development. Plant Mol. Biol. 53, 531–543.

Bendahmane, A., Kanyuka, K., Baulcombe, D.C., 1999. The Rx Gene from Potato Controls Separate Virus Resistance and Cell Death Responses. Plant Cell 11, 781–791.

Bendahmane, A., Querci, M., Kanyuka, K., Baulcombe, D.C., 2000. Agrobacterium transient expression system as a tool for the isolation of disease resistance genes: application to the Rx2 locus in potato. Plant J. 21, 73–81. https://doi.org/10.1046/j.1365-313x.2000.00654.x

Bengyella, L., Waikhom, S.D., Allie, F., Rey, C., 2015. Virus tolerance and recovery from viral induced-symptoms in plants are associated with transcriptome reprograming. Plant Mol. Biol. 89, 243–252. https://doi.org/10.1007/s11103-015-0362-6

Best, A., White, A., Boots, M., 2008. Maintenance of host variation in tolerance to pathogens and parasites. Proc. Natl. Acad. Sci. U. S. A. 105, 20786–91. https://doi.org/10.1073/pnas.0809558105

Bhattacharjee, S., Zamora, A., Azhar, M.T., Sacco, M.A., Lambert, L.H., Moffett, P., 2009. Virus resistance induced by NB-LRR proteins involves Argonaute4-dependent translational control. Plant J. 58, 940–951. https://doi.org/10.1111/j.1365-313X.2009.03832.x

Blevins, T., Rajeswaran, R., Shivaprasad, P. V., Beknazariants, D., Si-Ammour, A., Park, H.S., Vazquez, F., Robertson, D., Meins, F., Hohn, T., Pooggin, M.M., 2006. Four plant Dicers

mediate viral small RNA biogenesis and DNA virus induced silencing. Nucleic Acids Res. 34, 6233–6246.

Bohmert, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M., Benning, C., 1998. AGO1 defines a novel locus of Arabidopsis controlling leaf development. EMBO J. 17, 170–180.

Bologna, N.G., Voinnet, O., 2014. The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis. Annu. Rev. Plant Biol. 65. https://doi.org/10.1146/annurev-arplant-050213-035728

Borevitz, J.O., Hazen, S.P., Michael, T.P., Morris, G.P., Baxter, I.R., Hu, T.T., Chen, H., Werner, J.D., Nordborg, M., Salt, D.E., Kay, S.A., Chory, J., Weigel, D., Jones, J.D.G., Ecker, J.R., 2007. Genome-wide patterns of single-feature polymorphism in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 104, 12057–62. https://doi.org/10.1073/pnas.0705323104

Borges, F., Martienssen, R.A., 2015. The expanding world of small RNAs in plants. Nat. Publ. Gr. 16. https://doi.org/10.1038/nrm4085

Bortolamiol, D., Pazhouhandeh, M., Marrocco, K., Genschik, P., Ronique Ziegler-Graff, V., 2007. Report The Polerovirus F Box Protein P0 Targets ARGONAUTE1 to Suppress RNA Silencing. Curr. Biol. 17, 1615–1621. https://doi.org/10.1016/j.cub.2007.07.061

Boualem, A., Dogimont, C., Bendahmane, A., 2016. The battle for survival between viruses and their host plants. Curr. Opin. Virol. https://doi.org/10.1016/j.coviro.2015.12.001

Bouché, N., Lauressergues, D., Gasciolli, V., Vaucheret, H., 2006. An antagonistic function for Arabidopsis DCL2 in development and a new function for DCL4 in generating viral siRNAs. EMBO J. 25, 3347–3356. https://doi.org/10.1038/sj.emboj.7601217

Boutrot, F., Zipfel, C., 2017. Function, Discovery, and Exploitation of Plant Pattern Recognition Receptors for Broad-Spectrum Disease Resistance. Annu. Rev. Phytopathol. 55, 257–286. https://doi.org/10.1146/annurev-phyto-080614-120106

Boyes, D.C., Zayed, A.M., Ascenzi, R., Mccaskill, A.J., Hoffman, N.E., Davis, K.R., Görlach, J., 2001. Growth Stage-Based Phenotypic Analysis of Arabidopsis: A Model for High

Throughput Functional Genomics in Plants, The Plant Cell.

Brodersen, P., Sakvarelidze-Achard, L., Bruun-Rasmussen, M., Dunoyer, P., Yamamoto, Y.Y., Sieburth, L., Voinnet, O., 2008. Widespread Translational Inhibition by Plant miRNAs and siRNAs. Science (80-.). 320, 1185–1190. https://doi.org/10.1126/science.1159151

Brosseau, C., Adurogbangba, A., Roussin-Léveillée, C., Zhao, Z., Biga, S., Moffett, P., 2019. Natural variation in the Arabidopsis AGO2 gene is associated with susceptibility to potato virus X. bioRxiv 746628. https://doi.org/10.1101/746628

Brosseau, C., Moffett, P., 2015. Functional and Genetic Analysis Identify a Role for Arabidopsis ARGONAUTE5 in Antiviral RNA Silencing. Plant Cell 27, 1742–54. https://doi.org/10.1105/tpc.15.00264

Brosseau, C., Oirdi, M. El, Adurogbangba, A., Ma, X., Moffett, P., 2016. Antiviral Defense Involves AGO4 in an Arabidopsis–Potexvirus Interaction. MPMI 29, 878–888. https://doi.org/10.1094/MPMI-09-16-0188-R

Bucher, E., Lohuis, D., van Poppel, P.M.J.A., Geerts-Dimitriadou, C., Goldbach, R., Prins, M., 2006. Multiple virus resistance at a high frequency using a single transgene construct. J. Gen. Virol. 87, 3697–3701. https://doi.org/10.1099/vir.0.82276-0

Buchmann, R.C., Asad, S., Wolf, J.N., Mohannath, G., Bisaro, D.M., 2009. Geminivirus AL2 and L2 Proteins Suppress Transcriptional Gene Silencing and Cause Genome-Wide Reductions in Cytosine Methylation □. J. Virol. 83, 5005–5013. https://doi.org/10.1128/JVI.01771-08

Cao, J., Schneeberger, K., Ossowski, S., Günther, T., Bender, S., Fitz, J., Koenig, D., Lanz, C., Stegle, O., Lippert, C., Wang, X., Ott, F., Müller, J., Alonso-Blanco, C., Borgwardt, K., Schmid, K.J., Weigel, D., 2011. Whole-genome sequencing of multiple Arabidopsis thaliana populations. Nat. Genet. 43, 956–965. https://doi.org/10.1038/ng.911

Carbonell, A., Carrington, J.C., 2015. Antiviral roles of plant ARGONAUTES. Curr. Opin. Plant Biol. 27, 111–117. https://doi.org/10.1016/j.pbi.2015.06.013

Carbonell, A., Fahlgren, N., Garcia-Ruiz, H., Gilbert, K.B., Montgomery, T.A., Nguyen, T.,

Cuperus, J.T., Carrington, J.C., 2012. Functional analysis of three Arabidopsis ARGONAUTES using slicer-defective mutants. Plant Cell 24, 3613–29. https://doi.org/10.1105/tpc.112.099945

Carthew, R.W., Sontheimer, E.J., 2009. Origins and Mechanisms of miRNAs and siRNAs. Cell 136, 642–55. https://doi.org/10.1016/j.cell.2009.01.035

Chae, E., Tran, D.T.N., Weigel, D., 2016. Cooperation and Conflict in the Plant Immune System. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1005452

Chellappan, P., Vanitharani, R., Ogbe, F., Fauquet, C.M., 2005. Effect of temperature on geminivirus-induced RNA silencing in plants. Plant Physiol. 138, 1828–41. https://doi.org/10.1104/pp.105.066563

Chen, H., Zhang, L., Yu, K., Wang, A., 2015. Pathogenesis of Soybean mosaic virus in soybean carrying Rsv1 gene is associated with miRNA and siRNA pathways, and breakdown of AGO1 homeostasis. Virology 476, 395–404. https://doi.org/10.1016/j.virol.2014.12.034

Cheng, X., Wang, A., 2017. The Potyvirus Silencing Suppressor Protein VPg Mediates Degradation of SGS3 via Ubiquitination and Autophagy Pathways. J. Virol. 91. https://doi.org/10.1128/jvi.01478-16

Chisholm, S.T., Coaker, G., Day, B., Staskawicz, B.J., 2006. Host-microbe interactions: Shaping the evolution of the plant immune response. Cell.

Chiu, M.-H., Chen, I.-H., Baulcombe, D.C., Tsai, C.-H., 2010. The silencing suppressor P25 of Potato virus X interacts with Argonaute1 and mediates its degradation through the proteasome pathway. Mol. Plant Pathol. 11, 641–9. https://doi.org/10.1111/j.1364-3703.2010.00634.x

Cho, Y.B., Jones, S.I., Vodkin, L.O., 2017. Mutations in Argonaute5 Illuminate Epistatic Interactions of the K1 and I Loci Leading to Saddle Seed Color Patterns in Glycine max. Plant Cell 29, tpc.00162.2017. https://doi.org/10.1105/tpc.17.00162

Clark, R.M., Schweikert, G., Toomajian, C., Ossowski, S., Zeller, G., Shinn, P., Warthmann, N., Hu, T.T., Fu, G., Hinds, D.A., Chen, H., Frazer, K.A., Huson, D.H., Scholkopf, B.,

Nordborg, M., Ratsch, G., Ecker, J.R., Weigel, D., 2007. Common Sequence Polymorphisms Shaping Genetic Diversity in Arabidopsis thaliana. Science (80-.). 317, 338–342. https://doi.org/10.1126/science.1138632

Cook, D.E., Mesarich, C.H., Thomma, B.P.H.J., 2015. Understanding Plant Immunity as a Surveillance System to Detect Invasion. Annu. Rev. Phytopathol 53, 541–63. https://doi.org/10.1146/annurev-phyto-080614-120114

Cooley, M.B., Pathirana, S., Wu, H.J., Kachroo, P., Klessig, D.F., 2000. Members of the Arabidopsis HRT/RPP8 family of resistance genes confer resistance to both viral and oomycete pathogens. Plant Cell 12, 663–76. https://doi.org/10.1105/tpc.12.5.663

Cosson, P., Schurdi-Levraud, V., Le, Q.H., Sicard, O., Caballero, M., Roux, F., Le Gall, O., Candresse, T., Revers, F., 2012. The RTM resistance to potyviruses in Arabidopsis thaliana: natural variation of the RTM genes and evidence for the implication of additional genes. PLoS One 7, e39169. https://doi.org/10.1371/journal.pone.0039169

Csorba, T., Kontra, L., Burgyán, J., 2015. Viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. Virology. https://doi.org/10.1016/j.virol.2015.02.028

Csorba, T., Lózsa, R., Hutvágner, G., Burgyán, J., 2010. Polerovirus protein P0 prevents the assembly of small RNA-containing RISC complexes and leads to degradation of ARGONAUTE1. Plant J. 62, 463–472. https://doi.org/10.1111/j.1365-313X.2010.04163.x

Curtin, S.J., Michno, J.-M., Campbell, B.W., Gil-Humanes, J., Mathioni, S.M., Hammond, R., Gutierrez-Gonzalez, J.J., Donohue, R.C., Kantar, M.B., Eamens, A.L., Meyers, B.C., Voytas, D.F., Stupar, R.M., 2016. MicroRNA Maturation and MicroRNA Target Gene Expression Regulation Are Severely Disrupted in Soybean dicer-like1 Double Mutants. Genes, Genomes, Genet. 6, 423–433.

Dempsey, D.A., Klessig, D.F., 2012. SOS - too many signals for systemic acquired resistance? Trends Plant Sci.

Dennis Gonsalves, 2004. Transgenic Papaya in Hawaii and Beyond.

Derrien, B., Baumberger, N., Schepetilnikov, M., Viotti, C., De Cillia, J., Ziegler-Graff, V.,

Isono, E., Schumacher, K., Genschik, P., 2012. Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. Proc. Natl. Acad. Sci. 109, 15942–15946. https://doi.org/10.1073/pnas.1209487109

Di Serio, F., Schö, H., Iglesias, A., Tarina, C., Bouldoires, E., Meins, F., Phillips, R.L., 2001. Sense-and antisense-mediated gene silencing in tobacco is inhibited by the same viral suppressors and is associated with accumulation of small RNAs. PNAS 98, 6506–6510.

Diao, P., Zhang, Q., Sun, H., Ma, W., Cao, A., Yu, R., Wang, J., Niu, Y., Wuriyanghan, H., Diao, P., Zhang, Q., Sun, H., Ma, W., Cao, A., Yu, R., Wang, J., Niu, Y., Wuriyanghan, H., 2019. miR403a and SA Are Involved in NbAGO2 Mediated Antiviral Defenses Against TMV Infection in Nicotiana benthamiana. Genes (Basel). 10, 526. https://doi.org/10.3390/genes10070526

Dinesh-Kumar, S.P., Tham, W.-H., Baker, B.J., Gajiwala, P., Pasion, R., Whalen, M.C., Stall, R.E., Staskawicz, B.J., 2000. Structure-function analysis of the tobacco mosaic virus resistance gene N. Proc. Natl. Acad. Sci. 97, 14789–14794. https://doi.org/10.1073/pnas.97.26.14789

Donaire, L., Wang, Y., Gonzalez-Ibeas, D., Mayer, K.F., Aranda, M.A., Llave, C., 2009. Deep-sequencing of plant viral small RNAs reveals effective and widespread targeting of viral genomes. Virology 392, 203–214. https://doi.org/10.1016/j.virol.2009.07.005

Duan, C.-G., Fang, Y.-Y., Zhou, B.-J., Zhao, J.-H., Hou, W.-N., Zhu, H., Ding, S.-W., Guo, H.-S., 2012. Suppression of Arabidopsis ARGONAUTE1-Mediated Slicing, Transgene-Induced RNA Silencing, and DNA Methylation by Distinct Domains of the Cucumber mosaic virus 2b Protein. Plant Cell. https://doi.org/10.1105/tpc.111.092718

Duan, C.-G., Zhang, H., Tang, K., Zhu, X., Qian, W., Hou, Y.-J., Wang, B., Lang, Z., Zhao, Y., Wang, X., Wang, P., Zhou, J., Liang, G., Liu, N., Wang, C., Zhu, J.-K., 2015. Specific but interdependent functions for Arabidopsis AGO4 and AGO6 in RNA-directed DNA methylation. EMBO J. 34, 581–592. https://doi.org/10.15252/embj

Dunoyer, P., Schott, G., Himber, C., Meyer, D., Takeda, A., Carrington, J.C., Voinnet, O., 2010. Small RNA Duplexes Function as Mobile Silencing Signals Between Plant Cells. Science

(80-.). 328.

Dzianott, A., Sztuba-Solińska, J., Bujarski, J.J., 2012a. Mutations in the Antiviral RNAi Defense Pathway Modify Brome mosaic virus RNA Recombinant Profiles. Mol. Plant-Microbe Interact. 25, 97–106. https://doi.org/10.1094/MPMI-05-11-0137

Dzianott, A., Sztuba-Solińska, J., Bujarski, J.J., 2012b. Mutations in the Antiviral RNAi Defense Pathway Modify Brome mosaic virus RNA Recombinant Profiles 25, 97–106.

Eamens, A., Wang, M.-B., Smith, N.A., Waterhouse, P.M., 2008. RNA silencing in plants: yesterday, today, and tomorrow. Plant Physiol. 147, 456–68. https://doi.org/10.1104/pp.108.117275

El-Shami, M., Pontier, D., Lahmy, S., Braun, L., Picart, C., Vega, D., Hakimi, M.A., Jacobsen, S.E., Cooke, R., Lagrange, T., 2007. Reiterated WG/GW motifs form functionally and evolutionarily conserved ARGONAUTE-binding platforms in RNAi-related components. Genes Dev. 21, 2539–2544. https://doi.org/10.1101/gad.451207

El Oirdi, M., El Rahman, T.A., Rigano, L., El Hadrami, A., Rodriguez, M.C., Daayf, F., Vojnov, A., Bouarab, K., 2011. *Botrytis cinerea* Manipulates the Antagonistic Effects between Immune Pathways to Promote Disease Development in Tomato. Plant Cell 23, 2405–2421. https://doi.org/10.1105/tpc.111.083394

Elsharkawy, M.M., Shimizu, M., Takahashi, H., Ozaki, K., Hyakumachi, M., 2013. Induction of Systemic Resistance against Cucumber mosaic virus in Arabidopsis thaliana by Trichoderma asperellum SKT-1. Plant Pathol. J. 29, 193. https://doi.org/10.5423/PPJ.SI.07.2012.01

Enard, D., Cai, L., Gwennap, C., Petrov, D.A., 2016. Viruses are a dominant driver of protein adaptation in mammals. Elife 5, 1–25. https://doi.org/10.7554/eLife.12469

Eun, C., Lorkovic, Z.J., Naumann, U., Long, Q., Havecker, E.R., Simon, S.A., Meyers, B.C., Matzke, A.J.M., Matzke, M., Freitag, M., 2011. AGO6 Functions in RNA-Mediated Transcriptional Gene Silencing in Shoot and Root Meristems in Arabidopsis thaliana. https://doi.org/10.1371/journal.pone.0025730

Fagard, M., Boutet, S., Morel, J.-B., Bellini, C., Vaucheret, H., 2000. AGO1, QDE-2, and RDE-1 are required for post-transcriptional gene silencing in plants , quelling in fungi , and RNA interference in animals. Proc. Natl. Acad. Sci. 97, 11650–11654.

Fang, X., Qi, Y., 2016. RNAi in Plants: An Argonaute-Centered View. Plant Cell 28, 272–85. https://doi.org/10.1105/tpc.15.00920

Fang, Y.-Y., Zhao, J.-H., Liu, S.-W., Wang, S., Duan, C.-G., Guo, H.-S., 2016. CMV2b-AGO Interaction Is Required for the Suppression of RDR-Dependent Antiviral Silencing in Arabidopsis. Front. Microbiol. 7, 1329. https://doi.org/10.3389/fmicb.2016.01329

Fátyol, K., Ludman, M., Burgyán, J., P.D., Z., V., S., T., T., D.J., P., H., V., W.M., K., R., C., S., C., G.J., H., Y., Q., 2016. Functional dissection of a plant Argonaute. Nucleic Acids Res. 44, 1384–1397. https://doi.org/10.1093/nar/gkv1371

Feng, L., Duan, C.-G., Guo, H.-S., 2013. Inhibition of *in vivo* Slicer activity of Argonaute protein 1 by the viral 2b protein independent of its dsRNA-binding function. Mol. Plant Pathol. 14, 617–622. https://doi.org/10.1111/mpp.12033

Ferreira, S.A., Pitz, K.Y., 2002. Virus Coat Protein Transgenic Papaya Provides Practical Control of Papaya ringspot virus in Hawaii.

Frost, K.E., Groves, R.L., Charkowski, A.O., 2013. Integrated Control of Potato Pathogens Through Seed Potato Certification and Provision of Clean Seed Potatoes. https://doi.org/10.1094/PDIS-05-13-0477-FE

Fusaro, A.F., Matthew, L., Smith, N.A., Curtin, S.J., Dedic-Hagan, J., Ellacott, G.A., Watson, J.M., Wang, M.-B., Brosnan, C., Carroll, B.J., Waterhouse, P.M., 2006. RNA interference-inducing hairpin RNAs in plants act through the viral defence pathway. EMBO Rep. 7, 1168–75. https://doi.org/10.1038/sj.embor.7400837

Garcia-Ruiz, H., Carbonell, A., Hoyer, J.S., Fahlgren, N., Gilbert, K.B., Takeda, A., Giampetruzzi, A., Ruiz, M.T.G., Mcginn, M.G., Lowery, N., Martinez Baladejo, M.T., Carrington, J.C., -Ruiz, G., Carbonell, H., Js, H., Ding, B., 2015. Roles and Programming of Arabidopsis ARGONAUTE Proteins during Turnip Mosaic Virus Infection. PLoS Pathog

11. https://doi.org/10.1371/journal.ppat.1004755

Garcia-Ruiz, H., Takeda, A., Chapman, E.J., Sullivan, C.M., Fahlgren, N., Brempelis, K.J., Carrington, J.C., 2010. Arabidopsis RNA-Dependent RNA Polymerases and Dicer-Like Proteins in Antiviral Defense and Small Interfering RNA Biogenesis during Turnip Mosaic Virus Infection. Plant Cell 22, 481–496. https://doi.org/10.1105/tpc.109.073056

Gargouri-Bouzid, R., Jaoua, L., Rouis, S., Saídi, M.N., Bouaziz, D., Ellouz, R., 2006. PVY-Resistant Transgenic Potato Plants Expressing an Anti-NIa Protein scFv Antibody. Mol. Biotechnol. 33, 133–140. https://doi.org/10.1385/MB:33:2:133

Ghoshal, B., Sanfaçon, H., 2014. Temperature-dependent symptom recovery in Nicotiana benthamiana plants infected with tomato ringspot virus is associated with reduced translation of viral RNA2 and requires ARGONAUTE 1. Virology 456–457, 188–197. https://doi.org/10.1016/J.VIROL.2014.03.026

Gill, U.S., Lee, S., Mysore, K.S., 2015. Host Versus Nonhost Resistance: Distinct Wars with Similar Arsenals. Phytopathology. https://doi.org/10.1094/phyto-11-14-0298-rvw

Giner, A., Lakatos, L., García-Chapa, M., López-Moya, J.J., Burgyán, J., 2010. Viral Protein Inhibits RISC Activity by Argonaute Binding through Conserved WG/GW Motifs. PLoS Pathog. 6, e1000996. https://doi.org/10.1371/journal.ppat.1000996

Gonzalez-Gaitan, M., Jessell, T., Johnston, L., Laufer, E., McCabe, B., Struhl, G., Deleris, A., Gallego-Bartolome, J., Bao, J., Kasschau, K.D., Carrington, J.C., Voinnet, O., 2004. Hierarchical Action and Inhibition of Plant Dicer-Like Proteins in Antiviral Defense. Science (80-.). 305, 1462–322. https://doi.org/10.1126/science.1128650

González, I., Martínez, L., Rakitina, D. V., Lewsey, M.G., Atencio, F.A., Llave, C., Kalinina, N.O., Carr, J.P., Palukaitis, P., Canto, T., 2010. Cucumber mosaic virus 2b protein subcellular targets and interactions: Their significance to RNA silencing suppressor activity. Mol. Plant-Microbe Interact. 23, 294–303. https://doi.org/10.1094/MPMI-23-3-0294

González, I., Rakitina, D., Semashko, M., Taliansky, M., Praveen, S., Palukaitis, P., Carr, J.P., Kalinina, N., Canto, T., 2012. RNA binding is more critical to the suppression of silencing

function of Cucumber mosaic virus 2b protein than nuclear localization. RNA 18, 771–782. https://doi.org/10.1261/rna.031260.111

Goto, K., Kobori, T., Kosaka, Y., Natsuaki, T., Masuta, C., 2007. Characterization of Silencing Suppressor 2b of Cucumber Mosaic Virus Based on Examination of its Small RNA-Binding Abilities. Plant Cell Physiol. 48, 1050–1060. https://doi.org/10.1093/pcp/pcm074

Gross Juergen, L.U., 2015. Tests for Normality [R package nortest version 1.0-4].

Ha, M., Kim, V.N., 2014. Regulation of microRNA biogenesis. Nat. Rev. Mol. Cell Biol. 15, 509–524.

Haas, G., Azevedo, J., Moissiard, G., Le Geldreich, A., Himber, C., Bureau, M., Fukuhara, T., Keller, M., Voinnet, O., 2008. Nuclear import of CaMV P6 is required for infection and suppression of the RNA silencing factor DRB4. EMBO J. 27129, 2102–2112.

Hamera, S., Song, X., Su, L., Chen, X., Fang, R., 2012. Cucumber mosaic virus suppressor 2b binds to AGO4-related small RNAs and impairs AGO4 activities. Plant J. 69, 104–115.

Harvey, J.J.W., Lewsey, M.G., Patel, K., Westwood, J., Heimstädt, S., Carr, J.P., Baulcombe, D.C., 2011. An antiviral defense role of AGO2 in plants. PLoS One 6.

Hashimoto, M., Neriya, Y., Keima, T., Iwabuchi, N., Koinuma, H., Hagiwara-Komoda, Y., Ishikawa, K., Himeno, M., Maejima, K., Yamaji, Y., Namba, S., 2016. EXA1, a GYF domain protein, is responsible for loss-of-susceptibility to plantago asiatica mosaic virus in *Arabidopsis thaliana*. Plant J. 88, 120–131. https://doi.org/10.1111/tpj.13265

Hauptmann, J., Schraivogel, D., Bruckmann, A., Manickavel, S., Jakob, L., Eichner, N., Pfaff, J., Urban, M., Sprunck, S., Hafner, M., Tuschl, T., Deutzmann, R., Meister, G., 2015. Biochemical isolation of Argonaute protein complexes by Ago-APP. Proc. Natl. Acad. Sci. U. S. A. 112, 11841–5. https://doi.org/10.1073/pnas.1506116112

Havecker, E.R., Wallbridge, L.M., Hardcastle, T.J., Bush, M.S., Kelly, K.A., Dunn, R.M., Schwach, F., Doonan, J.H., Baulcombe, D.C., 2010. The Arabidopsis RNA-directed DNA methylation argonautes functionally diverge based on their expression and interaction with target loci. Plant Cell 22, 321–34. https://doi.org/10.1105/tpc.109.072199

Hernández-Lagana, E., Rodríguez-Leal, D., Lúa, J., Vielle-Calzada, J.P., 2016. A multigenic network of ARGONAUTE4 clade members controls early megaspore formation in arabidopsis. Genetics 204, 1045–1056. https://doi.org/10.1534/genetics.116.188151

Hily, J.M., García, A., Moreno, A., Plaza, M., Wilkinson, M.D., Fereres, A., Fraile, A., García-Arenal, F., 2014. The Relationship between Host Lifespan and Pathogen Reservoir Potential: An Analysis in the System Arabidopsis thaliana-Cucumber mosaic virus. PLoS Pathog. 10, e1004492. https://doi.org/10.1371/journal.ppat.1004492

Hohn, T., Vazquez, F., 2011. RNA silencing pathways of plants: Silencing and its suppression by plant DNA viruses ☆. BBA - Gene Regul. Mech. 1809, 588–600. https://doi.org/10.1016/j.bbagrm.2011.06.002

Howell, M.D., Fahlgren, N., Chapman, E.J., Cumbie, J.S., Sullivan, C.M., Givan, S.A., Kasschau, K.D., Carrington, J.C., 2007. Genome-wide analysis of the RNA-DEPENDENT RNA POLYMERASE6/DICER-LIKE4 pathway in Arabidopsis reveals dependency on miRNA- and tasiRNA-directed targeting. Plant Cell 19, 926–42. https://doi.org/10.1105/tpc.107.050062

Hu, P., Zhao, H., Zhu, P., Xiao, Y., Miao, W., Wang, Y., Jin, H., 2019. Dual regulation of Arabidopsis AGO2 by arginine methylation. Nat. Commun. 10, 844. https://doi.org/10.1038/s41467-019-08787-w

Hunter, C., 2006. Trans-acting siRNA-mediated repression of ETTIN and ARF4 regulates heteroblasty in Arabidopsis. Development 133, 2973–2981. https://doi.org/10.1242/dev.02491

Inoue-Nagata, A.K., Lima, M.F., Gilbertson, R.L., Inoue-Nagata, A.K., Lima, M.F., Gilbertson, R.L., 2016. A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. Hortic. Bras. 34, 8–18. https://doi.org/10.1590/S0102-053620160000100002

Ivanov, K.I., Eskelin, K., Bašić, M., De, S., Lõhmus, A., Varjosalo, M., Mäkinen, K., 2016a. Molecular insights into the function of the viral RNA silencing suppressor HCPro. Plant J. 85, 30–45. https://doi.org/10.1111/tpj.13088

Ivanov, K.I., Eskelin, K., Bašić, M., De, S., Lõhmus, A., Varjosalo, M., Mäkinen, K., 2016b. Molecular insights into the function of the viral RNA silencing suppressor HCPro. Plant J. 85, 30–45. https://doi.org/10.1111/tpj.13088

Ivanov, K.I., Eskelin, K., Lõ, A., Mä Kinen Correspondence, K., Mä, K., 2014. Molecular and cellular mechanisms underlying potyvirus infection. J. Gen. Virol. 95, 1415–1429. https://doi.org/10.1099/vir.0.064220-0

Iwasaki, S., Kobayashi, M., Yoda, M., Sakaguchi, Y., Katsuma, S., Suzuki, T., Tomari, Y., 2010. Hsc70/Hsp90 Chaperone Machinery Mediates ATP-Dependent RISC Loading of Small RNA Duplexes. Mol. Cell 39, 292–299. https://doi.org/10.1016/j.molcel.2010.05.015

Jacquemond, M., 2012. Cucumber Mosaic Virus. Adv. Virus Res. 84, 439–504. https://doi.org/10.1016/B978-0-12-394314-9.00013-0

Jain, R.K., Pappu, H.R., Pappu, S.S., Varma, A., Ram, R.D., 1998. Molecular characterisation of papaya ringspot potyvirus isolates from India. Ann. Appl. Biol. 132, 413–425. https://doi.org/10.1111/j.1744-7348.1998.tb05218.x

Jamous, R.M., Boonrod, K., Fuellgrabe, M.W., Ali-Shtayeh, M.S., Krczal, G., Wassenegger, M., 2011. The helper component-proteinase of the Zucchini yellow mosaic virus inhibits the Hua Enhancer 1 methyltransferase activity in vitro. J. Gen. Virol. 92, 2222–2226.

Jaubert, M., Bhattacharjee, S., Mello, A.F.S., Perry, K.L., Moffett, P., 2011. ARGONAUTE2 Mediates RNA-Silencing Antiviral Defenses against Potato virus X in Arabidopsis 1[W][OA]. Plant Physiol. 156. https://doi.org/10.1104/pp.111.178012

Jenner, C.E., Wang, X., Ponz, F., Walsh, J.A., 2002. A fitness cost for Turnip mosaic virus to overcome host resistance. Virus Res. 86, 1–6. https://doi.org/10.1016/S0168-1702(02)00031-X

Jens Tilsner, O.L., Louveaux, M., Ian M. Roberts, Sean N. Chapman, and Karl J. Oparka, 2013. Replication and trafficking of a plant virus are coupled at the entrances of plasmodesmata. J. Cell Biol 201, 981–995. https://doi.org/10.1083/jcb.201304003

Jones, J.D.G., Dangl, J.L., 2006. The plant immune system. Nature 444, 323–329.

Jones, J.D.G., Vance, R.E., Dangl, J.L., 2016. Intracellular innate immune surveillance devices in plants and animals. Science (80-.). 354, 1117. https://doi.org/10.1126/science.aaf6395

Jouannet, V., Moreno, A.B., Elmayan, T., Vaucheret, H., Crespi, M.D., Maizel, A., 2012. Cytoplasmic Arabidopsis AGO7 accumulates in membrane-associated siRNA bodies and is required for ta-siRNA biogenesis. EMBO J. 31, 1704–1713. https://doi.org/10.1038/emboj.2012.20

Kapoor, M., Arora, R., Lama, T., Nijhawan, A., Khurana, J.P., Tyagi, A.K., Kapoor, S., 2008. Genome-wide identification, organization and phylogenetic analysis of Dicer-like, Argonaute and RNA-dependent RNA Polymerase gene families and their expression analysis during reproductive development and stress in rice. BMC Genomics 9. https://doi.org/10.1186/1471-2164-9-451

Kasschau, K.D., Carrington, J.C., 1998. A counterdefensive strategy of plant viruses: Suppression of posttranscriptional gene silencing. Cell 95, 461–470. https://doi.org/10.1016/S0092-8674(00)81614-1

Keima, T., Hagiwara-Komoda, Y., Hashimoto, M., Neriya, Y., Koinuma, H., Iwabuchi, N., Nishida, S., Yamaji, Y., Namba, S., 2017. Deficiency of the eIF4E isoform nCBP limits the cell-to-cell movement of a plant virus encoding triple-gene-block proteins in Arabidopsis thaliana. Sci. Rep. 7, 1–13. https://doi.org/10.1038/srep39678

Kidner, C.A., Martienssen, R.A., 2005. The role of ARGONAUTE1 (AGO1) in meristem formation and identity. Dev. Biol. 280, 504–517. https://doi.org/10.1016/J.YDBIO.2005.01.031

Kim, V.N., 2005. Small RNAs: classification, biogenesis, and function. Mol. Cells 19, 1–15. https://doi.org/806 [pii]

Kirino, Y., Kim, N., de Planell-Saguer, M., Khandros, E., Chiorean, S., Klein, P.S., Rigoutsos, I., Jongens, T.A., Mourelatos, Z., 2009. Arginine methylation of Piwi proteins catalysed by dPRMT5 is required for Ago3 and Aub stability. Nat. Cell Biol. 11, 652–658. https://doi.org/10.1038/ncb1872

Kirino, Y., Vourekas, A., Sayed, N., de Lima Alves, F., Thomson, T., Lasko, P., Rappsilber, J., Jongens, T.A., Mourelatos, Z., 2010. Arginine methylation of Aubergine mediates Tudor binding and germ plasm localization. Rna 16, 70–78. https://doi.org/10.1261/rna.1869710

Koenig, D., Jimenez-Gomez, J.M., Kimura, S., Fulop, D., Chitwood, D.H., Headland, L.R., Kumar, R., Covington, M.F., Devisetty, U.K., Tat, A. V., Tohge, T., Bolger, A., Schneeberger, K., Ossowski, S., Lanz, C., Xiong, G., Taylor-Teeples, M., Brady, S.M., Pauly, M., Weigel, D., Usadel, B., Fernie, A.R., Peng, J., Sinha, N.R., Maloof, J.N., 2013. Comparative transcriptomics reveals patterns of selection in domesticated and wild tomato, Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.1309606110

Kontra, L., Csorba, T., Tavazza, M., Lucioli, A., Tavazza, R., Moxon, S., Tisza, V., Medzihradszky, A., Turina, M., Burgyán, J., 2016. Distinct Effects of p19 RNA Silencing Suppressor on Small RNA Mediated Pathways in Plants. PLoS Pathog. 12, e1005935. https://doi.org/10.1371/journal.ppat.1005935

Koornneef, M., Alonso-Blanco, C., Vreugdenhil, D., 2004. NATURALLY OCCURRING GENETIC VARIATION IN *ARABIDOPSIS THALIANA*. Annu. Rev. Plant Biol. 55, 141–172. https://doi.org/10.1146/annurev.arplant.55.031903.141605

Kosakovsky Pond, S.L., Frost, S.D.W., 2005. Not So Different After All: A Comparison of Methods for Detecting Amino Acid Sites Under Selection. Mol. Biol. Evol. 22, 1208–1222. https://doi.org/10.1093/molbev/msi105

Kurihara, Y., Watanabe, Y., 2004. Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. PNAS 101, 12753–12758.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685. https://doi.org/10.1038/227680a0

Lakatos, L., Szittya, G., Silhavy, D., Burgyán, J., 2004. Molecular mechanism of RNA silencing suppression mediated by p19 protein of tombusviruses. EMBO J. 23, 876–884.

Lario, L.D., Ramirez-Parra, E., Gutierrez, C., Spampinato, C.P., Casati, P., 2013. ANTI-

SILENCING FUNCTION1 Proteins Are Involved in Ultraviolet-Induced DNA Damage Repair and Are Cell Cycle Regulated by E2F Transcription Factors in Arabidopsis 1[C][W][OA]. Plant Physiol. 162, 1164–1177. https://doi.org/10.1104/pp.112.212837

Lau, W., Fischbach, M.A., Osbourn, A., Sattely, E.S., 2014. Key applications of plant metabolic engineering. PLoS Biol. 12, e1001879. https://doi.org/10.1371/journal.pbio.1001879

Laufs, J., Trautt, W., Heyraudt, F., Matzeitt, V., Rogersf, S.G., Schellt, J., Gronenborn, B., 1995. In vitro cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. Biochemistry 92, 3879–3883.

Laura R. Newburn, K.A.W., 2015. Cis-acting RNA elements in positive-strand RNA plant virus genomes. Virology 479, 434–443.

Lee, S.-C., Wu, C.-H., Wang, C.-W., 2010. Traffic of a Viral Movement Protein Complex to the Highly Curved Tubules of the Cortical Endoplasmic Reticulum. Traffic 11, 912–930. https://doi.org/10.1111/j.1600-0854.2010.01064.x

Lee, S., Rojas, C.M., Ishiga, Y., Pandey, S., Mysore, K.S., 2013. Arabidopsis heterotrimeric G-proteins play a critical role in host and nonhost resistance against Pseudomonas syringae pathogens. PLoS One 8, e82445. https://doi.org/10.1371/journal.pone.0082445

Lee, S., Whitaker, V.M., Hutton, S.F., 2016. Mini Review: Potential Applications of Non-host Resistance for Crop Improvement. Front. Plant Sci. https://doi.org/10.3389/fpls.2016.00997

Li, C.F., Henderson, I.R., Song, L., Fedoroff, N., Lagrange, T., Jacobsen, S.E., 2008. Dynamic regulation of ARGONAUTE4 within multiple nuclear bodies in Arabidopsis thaliana. PLoS Genet. 4. https://doi.org/10.1371/journal.pgen.0040027

Li, C.F., Pontes, O., El-Shami, M., Henderson, I.R., Bernatavichute, Y. V., Chan, S.W.L., Lagrange, T., Pikaard, C.S., Jacobsen, S.E., 2006. An ARGONAUTE4-Containing Nuclear Processing Center Colocalized with Cajal Bodies in Arabidopsis thaliana. Cell 126, 93–106. https://doi.org/10.1016/j.cell.2006.05.032

Li, F., Wang, A., 2019. RNA-Targeted Antiviral Immunity: More Than Just RNA Silencing. Trends Microbiol. xx, 1–14. https://doi.org/10.1016/j.tim.2019.05.007

Li, F., Zhao, N., Li, Z., Xu, X., Wang, Y., Yang, X., Liu, S.-S., Wang, A., Zhou, X., 2017. A calmodulin-like protein suppresses RNA silencing and promotes geminivirus infection by degrading SGS3 via the autophagy pathway in Nicotiana benthamiana. PLoS Pathog. 13, e1006213. https://doi.org/10.1371/journal.ppat.1006213

Liang Wu, Huan Chen, C.C.& Z.Q.F., 2014. Go in for the kill: How plants deploy effectortriggered immunity to combat pathogens. Virulence 5, 710–721. https://doi.org/10.4161/viru.29755

Liao, Y.-C., Pingli, H., Senzhao, C., Yao, M.-J., Zhang, J.-B., Liu, J.-L., Lia0, Y.-C., 2006. Biotechnology and Genetic Engineering Reviews Plantibodies: A Novel Strategy to Create Pathogen-Resistant Plants 13 Plantibodies: A Novel Strategy to Create Pathogen-Resistant Plants. Biotechnol Genet Eng Rev. 23, 253–71. https://doi.org/10.1080/02648725.2006.10648087

Lico, C., Benvenuto, E., Baschieri, S., 2015. The Two-Faced Potato Virus X: From Plant Pathogen to Smart Nanoparticle. Front. Plant Sci. 6, 1009. https://doi.org/10.3389/fpls.2015.01009

Liu, J., Carmell, M. a, Rivas, F. V, Marsden, C.G., Thomson, J.M., Song, J.-J., Hammond, S.M., Joshua-Tor, L., Hannon, G.J., 2004. Argonaute2 is the catalytic engine of mammalian RNAi. Science 305, 1437–1441. https://doi.org/10.1126/science.1102513

Liu, Q., Feng, Y., Zhu, Z., 2009. Dicer-like (DCL) proteins in plants. Funct. Integr. Genomics.

Lough, T.J., Lee, R.H., Emerson, S.J., Forster, R.L.S., Lucas, W.J., 2006. Functional analysis of the 5′ untranslated region of potexvirus RNA reveals a role in viral replication and cell-to-cell movement. Virology 351, 455–465. https://doi.org/10.1016/J.VIROL.2006.03.043

Lu, R., Malcuit, T., Moffett, P., Ruiz, M.T., Peart, J., Wu, A.J., Rathjen, J.P., Bendahmane, A., Day, L., Baulcombe, D.C., 2003. High throughput virus-induced gene silencing implicates heat shock protein 90 in plant disease resistance. Embo J 22. https://doi.org/10.1093/emboj/cdg546

Lucht, J.M., 2015. Public Acceptance of Plant Biotechnology and GM Crops. Viruses 7, 4254–

81. https://doi.org/10.3390/v7082819

Ludman, M., Burgyán, J., Fátyol, K., 2017a. Crispr/Cas9 Mediated Inactivation of Argonaute 2 Reveals its Differential Involvement in Antiviral Responses. Sci. Rep. 7, 1010. https://doi.org/10.1038/s41598-017-01050-6

Ludman, M., Burgyán, J., Fátyol, K., 2017b. Crispr/Cas9 Mediated Inactivation of Argonaute 2 Reveals its Differential Involvement in Antiviral Responses /631/337/384/2053 /631/449/2169/597 /42/89 /42/44 /38/22 article. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-01050-6

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P., Barton, M.K., 1999. The PINHEAD / ZWILLE gene acts pleiotropically in Arabidopsis development and has overlapping functions with the ARGONAUTE1 gene. Development 481, 469–481.

Ma, J.-B., Ye, K., Patel, D.J., 2004. Structural basis for overhang-specific small interfering RNA recognition by the PAZ domain. Nature 429, 318–22. https://doi.org/10.1038/nature02519

Ma, X., Nicole, M.-C., Meteignier, L.-V., Hong, N., Wang, G., Moffett, P., 2015. Different roles for RNA silencing and RNA processing components in virus recovery and virus-induced gene silencing in plants. J. Exp. Bot. 66, 919–932. https://doi.org/10.1093/jxb/eru447

Mallory, A., Vaucheret, H., 2010. Form, function, and regulation of ARGONAUTE proteins. Plant Cell 22, 3879–89. https://doi.org/10.1105/tpc.110.080671

Mallory, A.C., Hinze, A., Tucker, M.R., Bouche, N., Gasciolli, V., Elmayan, T., Lauressergues, D., Jauvion, V., Vaucheret, H., Laux, T., 2009. Redundant and Specific Roles of the ARGONAUTE Proteins AGO1 and ZLL in Development and Small RNA- Directed Gene Silencing. PLoS Genet. 5, 1–12. https://doi.org/10.1371/journal.pgen.1000646

Manavella, P.A., Koenig, D., Weigel, D., 2012. Plant secondary siRNA production determined by microRNA-duplex structure. Proc. Natl. Acad. Sci. U. S. A. 109, 2461–6.

Manzano-Piedras, E., Marcer, A., Alonso-Blanco, C., Picó, F.X., 2014. Deciphering the Adjustment between Environment and Life History in Annuals: Lessons from a Geographically-Explicit Approach in Arabidopsis thaliana. PLoS One 9, e87836.

https://doi.org/10.1371/journal.pone.0087836

Matić, S., Noris, E., Contin, R., Marian, D., Thompson, J.R., 2019. Engineering partial resistance to cucumber mosaic virus in tobacco using intrabodies specific for the viral polymerase. Phytochemistry 162, 99–108. https://doi.org/10.1016/J.PHYTOCHEM.2019.03.006

Matzke, M. a, Kanno, T., Matzke, A.J.M., 2014. RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants. Annu. Rev. Plant Biol. 1–25. https://doi.org/10.1146/annurev-arplant-043014-114633

Matzke, M.A., Kanno, T., Matzke, A.J.M., 2015. RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants. Annu. Rev. Plant Biol. 66, 243–267. https://doi.org/10.1146/annurev-arplant-043014-114633

Maunoury, N., Vaucheret, H., 2011. AGO1 and AGO2 Act Redundantly in miR408-Mediated Plantacyanin Regulation. PLoS One 6, e28729. https://doi.org/10.1371/journal.pone.0028729

Mauricio, R., 1998. Costs of Resistance to Natural Enemies in Field Populations of the Annual Plant Arabidopsis thaliana Costs of Resistance to Natural Enemies in Field Populations of the Annual Plant Arabidopsis thaliana count for the common observation that, although plant. Source Am. Nat. 151, 20–28. https://doi.org/10.1086/286099

McCue, A.D., Panda, K., Nuthikattu, S., Choudury, S.G., Thomas, E.N., Slotkin, R.K., 2015. ARGONAUTE 6 bridges transposable element mRNA-derived siRNAs to the establishment of DNA methylation. EMBO J. 34, 20–35. https://doi.org/10.15252/embj.201489499

McHale, L., Tan, X., Koehl, P., Michelmore, R.W., 2006. Plant NBS-LRR proteins: adaptable guards. Genome Biol. 7, 212. https://doi.org/10.1186/gb-2006-7-4-212

Meister, G., 2013. Argonaute proteins: Functional insights and emerging roles. Nat. Rev. Genet. https://doi.org/10.1038/nrg3462

Méndez-Vigo, B., Gomaa, N.H., Alonso-Blanco, C., Xavier Picó, F., 2013. Among- and within-population variation in flowering time of Iberian *Arabidopsis thaliana* estimated in field and glasshouse conditions. New Phytol. 197, 1332–1343. https://doi.org/10.1111/nph.12082

Méndez-Vigo, B., Picó, F.X., Ramiro, M., Martínez-Zapater, J.M., Alonso-Blanco, C., 2011. Altitudinal and Climatic Adaptation Is Mediated by Flowering Traits and FRI, FLC, and PHYC Genes in Arabidopsis. Plant Physiol. 157, 1942–1955. https://doi.org/10.1104/PP.111.183426

Mérai, Z., Kerényi, Z., Molnár, A., Barta, E., Válóczi, A., Bisztray, G., Havelda, Z., Burgyán, J., Silhavy, D., 2005. Aureusvirus P14 is an efficient RNA silencing suppressor that binds double-stranded RNAs without size specificity. J. Virol. 79, 7217–26. https://doi.org/10.1128/JVI.79.11.7217-7226.2005

Mi, S., Cai, T., Hu, Y., Chen, Y., Hodges, E., Ni, F., Wu, L., Li, S., Zhou, H., Long, C., Chen, S., Hannon, G.J., Qi, Y., 2008. Sorting of Small RNAs into Arabidopsis Argonaute Complexes Is Directed by the 5′ Terminal Nucleotide. Cell 133, 116–127. https://doi.org/10.1016/j.cell.2008.02.034

Mingot, A., Valli, A., Rodamilans, B., San León, D., Baulcombe, D.C., García, J.A., López-Moya, J.J., 2016. The P1N-PISPO trans -Frame Gene of Sweet Potato Feathery Mottle Potyvirus Is Produced during Virus Infection and Functions as an RNA Silencing Suppressor . J. Virol. 90, 3543–3557. https://doi.org/10.1128/jvi.02360-15

Miyashita, Y., Atsumi, G., Nakahara, K.S., 2016. Trade-Offs for Viruses in Overcoming Innate Immunities in Plants. Mol. Plant-Microbe Interact. 29, 595–598. https://doi.org/10.1094/MPMI-05-16-0103-CR

Mlotshwa, S., Pruss, G.J., Vance, V., 2008. Small RNAs in viral infection and host defense. Trends Plant Sci. 13, 375–382. https://doi.org/10.1016/j.tplants.2008.04.009

Molnar, A., Melnyk, C.W., Bassett, A., Hardcastle, T.J., Dunn, R., Baulcombe, D.C., 2010. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science 328, 872–875. https://doi.org/10.1126/science.1187959

Montavon, T., Kwon, Y., Zimmermann, A., Hammann, P., Vincent, T., Cognat, V., Michel, F., Dunoyer, P., 2017. A specific dsRNA-binding protein complex selectively sequesters endogenous inverted-repeat siRNA precursors and inhibits their processing. Nucleic Acids Res. 45, 1330–1344. https://doi.org/10.1093/nar/gkw1264

Montes, N., Alonso-Blanco, C., García-Arenal, F., 2019. Cucumber mosaic virus infection as a potential selective pressure on Arabidopsis thaliana populations. PLOS Pathog. 15, e1007810. https://doi.org/10.1371/journal.ppat.1007810

Montgomery, T.A., Howell, M.D., Cuperus, J.T., Li, D., Hansen, J.E., Alexander, A.L., Chapman, E.J., Fahlgren, N., Allen, E., Carrington, J.C., 2008. Specificity of ARGONAUTE7-miR390 Interaction and Dual Functionality in TAS3 Trans-Acting siRNA Formation. Cell 133, 128–141. https://doi.org/10.1016/j.cell.2008.02.033

Morel, J., Godon, C., Mourrain, P., Béclin, C., Boutet, S., Feuerbach, F., Proux, F., Vaucheret, H., 2002. Fertile Hypomorphic *ARGONAUTE* (*ago1*) mutants impaired in post-transcriptional gene silencing and virus resistance. Society 14, 629–639. https://doi.org/10.1105/tpc.010358.are

Moreno, A.B., Martínez De Alba, A.E., Bardou, F., Crespi, M.D., Vaucheret, H., Maizel, A., Mallory, A.C., 2013. Cytoplasmic and nuclear quality control and turnover of single-stranded RNA modulate post-transcriptional gene silencing in plants. Nucleic Acids Res. 41, 4699–4708. https://doi.org/10.1093/nar/gkt152

Mourrain, P., Bé, C., Elmayan, T., Feuerbach, F., Godon, C., Morel, J.-B., Jouette, D., Lacombe, A.-M., Nikic, S., Picault, N., Ré, K., Sanial, M., Vo, T.-A., Vaucheret, H., 2000. Arabidopsis SGS2 and SGS3 Genes Are Required for Posttranscriptional Gene Silencing and Natural Virus Resistance gene required for PTGS, a possible mechanistic link. Cell 101, 533–542.

Moussian, B., Schoof, H., Haecker, A., Jurgens, G., Laux, T., 1998. Role of the ZWILLE gene in the regulation of central shoot meristem cell fate during Arabidopsis embryogenesis. EMBO J. 17, 1799–1809.

Muangsan, N., Beclin, C., Vaucheret, H., Robertson, D., 2004. Geminivirus VIGS of endogenous genes requires SGS2/SDE1 and SGS3 and defines a new branch in the genetic pathway for silencing in plants. Plant J. 38, 1004–1014. https://doi.org/10.1111/j.1365-313X.2004.02103.x

Mukherjee, K., Campos, H., Kolaczkowski, B., 2013. Evolution of animal and plant dicers: Early

parallel duplications and recurrent adaptation of antiviral RNA binding in plants. Mol. Biol. Evol. 30, 627–641. https://doi.org/10.1093/molbev/mss263

Musiyenko, A., Majumdar, T., Andrews, J., Adams, B., Barik, S., 2012. PRMT1 methylates the single Argonaute of Toxoplasma gondii and is important for the recruitment of Tudor nuclease for target RNA cleavage by antisense guide RNA. Cell. Microbiol. 14, 882–901. https://doi.org/10.1111/j.1462-5822.2012.01763.x

Nakasugi, K., Crowhurst, R.N., Bally, J., Wood, C.C., Hellens, R.P., Waterhouse, P.M., 2013. De novo transcriptome sequence assembly and analysis of RNA silencing genes of Nicotiana benthamiana. PLoS One 8, e59534. https://doi.org/10.1371/journal.pone.0059534

Naylor, M., Murphy, A.M., Berry, J.O., Carr, J.P., 1998. Salicylic Acid Can Induce Resistance to Plant Virus Movement 11, 860–868.

Nguyen, H.D., Tomitaka, Y., Ho, S.Y.W., Duchêne, S., Vetten, H.-J., Lesemann, D., Walsh, J.A., Gibbs, A.J., Ohshima, K., 2013. Turnip Mosaic Potyvirus Probably First Spread to Eurasian Brassica Crops from Wild Orchids about 1000 Years Ago. PLoS One 8, e55336. https://doi.org/10.1371/journal.pone.0055336

Noah Fahlgren, T.A.M., Miya D. Howell,Edwards Allen, Sarah K. Dvorak, Amanda L. Alexander, and J.C.C., 2006. Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA Affects Developmental Timing and Patterning in Arabidopsis. Curr. Biol. 16, 939–944. https://doi.org/10.1016/j.cub.2006.03.065

Nonomura, K.-I., Morohoshi, A., Nakano, M., Eiguchi, M., Miyao, A., Hirochika, H., Kurata, N., 2007. A Germ Cell–Specific Gene of the ARGONAUTE Family Is Essential for the Progression of Premeiotic Mitosis and Meiosis during Sporogenesis in Rice. Plant Cell 19, 2583–2594. https://doi.org/10.1105/tpc.107.053199

Nordborg, M., Hu, T.T., Ishino, Y., Jhaveri, J., Toomajian, C., Zheng, H., Bakker, E., Calabrese, P., Gladstone, J., Goyal, R., Jakobsson, M., Kim, S., Morozov, Y., Padhukasahasram, B., Plagnol, V., Rosenberg, N.A., Shah, C., Wall, J.D., Wang, J., Zhao, K., Kalbfleisch, T., Schulz, V., Kreitman, M., Bergelson, J., 2005. The pattern of polymorphism in Arabidopsis thaliana. PLoS Biol. 3, 1289–1299. https://doi.org/10.1371/journal.pbio.0030196

Nuthikattu, S., McCue, A.D., Panda, K., Fultz, D., DeFraia, C., Thomas, E.N., Slotkin, R.K., 2013. The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21-22 nucleotide small interfering RNAs. Plant Physiol. 162, 116–31. https://doi.org/10.1104/pp.113.216481

Oa, W., Garcia-Ruiz, H., Takeda, A., Chapman, E.J., Sullivan, C.M., Fahlgren, N., Brempelis, K.J., Carrington, J.C., 2010. Arabidopsis RNA-Dependent RNA Polymerases and Dicer-Like Proteins in Antiviral Defense and Small Interfering RNA Biogenesis during Turnip Mosaic Virus Infection. Plant Cell 22, 481–496. https://doi.org/10.1105/tpc.109.073056

Obbard, D.J., Jiggins, F.M., Halligan, D.L., Little, T.J., 2006. Natural selection drives extremely rapid evolution in antiviral RNAi genes. Curr. Biol. 16, 580–585. https://doi.org/10.1016/j.cub.2006.01.065

Odokonyero, D., Mendoza, M.R., Alvarado, V.Y., Zhang, J., Wang, X., Scholthof, H.B., 2015. Transgenic down-regulation of ARGONAUTE2 expression in Nicotiana benthamiana interferes with several layers of antiviral defenses. Virology 486, 209–218. https://doi.org/10.1016/j.virol.2015.09.008

Odokonyero, D., Mendoza, M.R., Moffett, P., Scholthof, H.B., 2017. *Tobacco rattle virus* (TRV)-Mediated Silencing of *Nicotiana benthamiana ARGONAUTES* (*NbAGOs*) Reveals New Antiviral Candidates and Dominant Effects of TRV- *NbAGO1*. Phytopathology PHYTO-02-17-004. https://doi.org/10.1094/PHYTO-02-17-0049-R

Okano, Y., Senshu, H., Hashimoto, M., Neriya, Y., Netsu, O., Minato, N., Yoshida, T., Maejima, K., Oshima, K., Komatsu, K., Yamaji, Y., Namba, S., 2014. In Planta Recognition of a Double-Stranded RNA Synthesis Protein Complex by a Potexviral RNA Silencing Suppressor. Plant Cell 26, 2168–2183. https://doi.org/10.1105/tpc.113.120535

Olmedo-Monfil, V., Durán-Figueroa, N., Arteaga-Vázquez, M., Demesa-Arévalo, E., Autran, D., Grimanelli, D., Slotkin, R.K., Martienssen, R.A., Vielle-Calzada, J.-P., 2010. Control of female gamete formation by a small RNA pathway in Arabidopsis. Nature 464, 628–632. https://doi.org/10.1038/nature08828

Olspert, A., Chung, B.Y., Atkins, J.F., Carr, J.P., Firth, A.E., 2015. Transcriptional slippage in

the positive-sense RNA virus family Potyviridae. EMBO Rep. 16, 995–1004. https://doi.org/10.15252/embr.201540509

Omarov, R.T., Ciomperlik, J., Scholthof, H.B., 2016. An in vitro reprogrammable antiviral RISC with size-preferential ribonuclease activity. Virology 490, 41–48. https://doi.org/10.1016/J.VIROL.2015.12.017

Pagán, I., Alonso-Blanco, C., García-Arenal, F., 2009. Differential Tolerance to Direct and Indirect Density-Dependent Costs of Viral Infection in Arabidopsis thaliana. PLoS Pathog. 5, e1000531. https://doi.org/10.1371/journal.ppat.1000531

Pagán, I., Alonso-Blanco, C., García-Arenal, F., 2008. Host Responses in Life-History Traits and Tolerance to Virus Infection in Arabidopsis thaliana. PLoS Pathog. 4, e1000124. https://doi.org/10.1371/journal.ppat.1000124

Pagán, I., Fraile, A., Fernandez-Fueyo, E., Montes, N., Alonso-Blanco, C., García-Arenal, F., 2010a. Arabidopsis thaliana as a model for the study of plant-virus co-evolution. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 365, 1983–95. https://doi.org/10.1098/rstb.2010.0062

Pagán, I., Fraile, A., Fernandez-Fueyo, E., Montes, N., Alonso-Blanco, C., García-Arenal, F., 2010b. Arabidopsis thaliana as a model for the study of plant–virus co-evolution. Philos. Trans. R. Soc. B Biol. Sci. 365, 1983–1995. https://doi.org/10.1098/rstb.2010.0062

Pagán, I., García-Arenal, F., 2018. Tolerance to Plant Pathogens: Theory and Experimental Evidence. Int. J. Mol. Sci. 19, 810. https://doi.org/10.3390/ijms19030810

Palukaitis, P., Roossinck, M.J., Dietzgen, R.G., Francki, R.I.B., 1992. Cucumber MOSAIC Virus. Adv. Virus Res. 41, 281–348. https://doi.org/10.1016/S0065-3527(08)60039-1

Parent, J.-S., Bouteiller, N., Elmayan, T., Vaucheret, H., 2015. Respective contributions of Arabidopsis DCL2 and DCL4 to RNA silencing. Plant J. 81, 223–232. https://doi.org/10.1111/tpj.12720

Paudel, D.B., Ghoshal, B., Jossey, S., Ludman, M., Fatyol, K., Sanfaçon, H., 2018. Expression and antiviral function of ARGONAUTE 2 in Nicotiana benthamiana plants infected with two isolates of tomato ringspot virus with varying degrees of virulence. Virology 524, 127–

139. https://doi.org/10.1016/J.VIROL.2018.08.016

Paudel, D.B., Sanfaçon, H., 2018. Exploring the Diversity of Mechanisms Associated With Plant Tolerance to Virus Infection. Front. Plant Sci. 9, 1575. https://doi.org/10.3389/fpls.2018.01575

Paulo, J., Machado, B., Calil, I.P., Santos, A.A., Fontes, E.P.B., 2017. Translational control in plant antiviral immunity. Genet. Mol. Biol. 40, 292–304. https://doi.org/10.1590/1678-4685-GMB-2016-0092

Peart, J.R., Cook, G., Feys, B.J., Parker, J.E., Baulcombe, D.C., 2002a. An EDS1 orthologue is required for N-mediated resistance against tobacco mosaic virus. Plant J. 29, 569–579. https://doi.org/10.1046/j.1365-313X.2002.029005569.x

Peart, J.R., Lu, R., Sadanandom, A., Malcuit, I., Moffett, P., Brice, D.C., Schauser, L., Jaggard, D.A.W., Xiao, S., Coleman, M.J., Dow, M., Jones, J.D.G., Shirasu, K., Baulcombe, D.C., 2002b. Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. Proc. Natl. Acad. Sci. U. S. A. 99, 10865–10869. https://doi.org/10.1073/pnas.152330599

Picó, F.X., Méndez-Vigo, B., Martínez-Zapater, J.M., Alonso-Blanco, C., 2008. Natural Genetic Variation of Arabidopsis thaliana Is Geographically Structured in the Iberian Peninsula. Genetics 180, 1009–1021. https://doi.org/10.1534/genetics.108.089581

Pieterse, C.M.J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., Van Wees, S.C.M., 2012. Hormonal Modulation of Plant Immunity. Annu. Rev. Cell Dev. Biol. 28, 489–521. https://doi.org/10.1146/annurev-cellbio-092910-154055

Pita, J.S., Morris, V., Roossinck, M.J., 2015. Mutation and Recombination Frequencies Reveal a Biological Contrast within Strains of Cucumber Mosaic Virus Downloaded from. J Virol 89, 6817–6823. https://doi.org/10.1128/JVI.01891-12

Pogany, J., Nagy, P.D., 2015. Activation of *Tomato Bushy Stunt Virus* RNA-Dependent RNA Polymerase by Cellular Heat Shock Protein 70 Is Enhanced by Phospholipids *In Vitro*. J. Virol. 89, 5714–5723. https://doi.org/10.1128/JVI.03711-14

Pogany, J., Nagy, P.D., 2012. p33-Independent Activation of a Truncated p92 RNA-Dependent RNA Polymerase of Tomato Bushy Stunt Virus in Yeast Cell-Free Extract. J. Virol. 86, 12025–12038.

Pontier, D., Picart, C., Roudier, F.O., Garcia, D., Lahmy, S., Azevedo, J., Alart, E., Le Laudié, M., Karlowski, W.M., Cooke, R., Colot, V., Voinnet, O., Lagrange, T., 2012. Molecular Cell Article NERD, a Plant-Specific GW Protein, Defines an Additional RNAi-Dependent Chromatin-Based Pathway in Arabidopsis. https://doi.org/10.1016/j.molcel.2012.07.027

Poulsen, C., Vaucheret, H., Brodersen, P., 2013. Lessons on RNA silencing mechanisms in plants from eukaryotic argonaute structures. Plant Cell 25, 22–37. https://doi.org/10.1105/tpc.112.105643

Powers, J.G., Sit, T.L., Qu, F., Morris, T.J., Kim, K.-H., Lommel, S.A., 2008. A Versatile Assay for the Identification of RNA Silencing Suppressors Based on Complementation of Viral Movement 21, 879–890.

Pradhan, M., Pandey, P., Gase, K., Sharaff, M., Singh, R., Sethi, A., Baldwin, I.T., Pandey, S.P., 2017. AGO8 mediates defense response against herbivores 2. Plant Physiol. https://doi.org/10.1104/pp.17.00702

Pumplin, N., Voinnet, O., 2013. RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence. Nat. Rev. Microbiol. 11, 745–60.

Qi, X., Bao, F.S., Xie, Z., 2009. Small RNA deep sequencing reveals role for Arabidopsis thaliana RNA-dependent RNA polymerases in viral siRNA biogenesis. PLoS One 4, e4971. https://doi.org/10.1371/journal.pone.0004971

Qi, Y., Denli, A.M., Hannon, G.J., 2005. Technique Biochemical Specialization within Arabidopsis RNA Silencing Pathways. Mol. Cell 19, 421–428. https://doi.org/10.1016/j.molcel.2005.06.014

Qu, F., Morris, T.J., 2005. Suppressors of RNA silencing encoded by plant viruses and their role in viral infections. FEBS Lett.

Qu, F., Ye, X., Morris, T.J., 2008. Arabidopsis DRB4, AGO1, AGO7, and RDR6 participate in a

DCL4-initiated antiviral RNA silencing pathway negatively regulated by DCL1. Proc. Natl. Acad. Sci. U. S. A. 105, 14732–7. https://doi.org/10.1073/pnas.0805760105

Raja, Priya, Jackel, J.N., Li, S., Heard, I.M., Bisaro, D.M., 2014. Arabidopsis double-stranded RNA binding protein DRB3 participates in methylation-mediated defense against geminiviruses. J. Virol. 88, 2611–22. https://doi.org/10.1128/JVI.02305-13

Raja, P., Jackel, J.N., Li, S., Heard, I.M., Bisaro, D.M., 2014. Arabidopsis Double-Stranded RNA Binding Protein DRB3 Participates in Methylation-Mediated Defense against Geminiviruses. J. Virol. 88, 2611–2622. https://doi.org/10.1128/JVI.02305-13

Raja, P., Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008a. Viral Genome Methylation as an Epigenetic Defense against Geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, Priya, Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008. Viral genome methylation as an epigenetic defense against geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, P., Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008b. Viral Genome Methylation as an Epigenetic Defense against Geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, P., Wolf, J.N., Bisaro, D.M., 2010. RNA silencing directed against geminiviruses: Post-transcriptional and epigenetic components. Biochim. Biophys. Acta - Gene Regul. Mech. 1799, 337–351. https://doi.org/10.1016/j.bbagrm.2010.01.004

Rambod Abiri, al, Valdiani, A., Maziah, M., Azmi Shaharuddin, N., Sahebi, M., Norhana Balia Yusof, Z., Atabaki, N., Talei, D., Valdiani alireza, A., 2016. A Critical Review of the Concept of Transgenic Plants: Insights into Pharmaceutical Biotechnology and Molecular Farming. Curr. Issues Mol. Biol. 18, 21–42. https://doi.org/10.21775/cimb.018.021

Reinhart, B.J., Weinstein, E.G., Rhoades, M.W., Bartel, B., Bartel, D.P., 2002. MicroRNAs in plants. Genes Dev.

Revers, F., García, J.A., 2015a. Molecular biology of potyviruses, in: Advances in Virus

Research. Academic Press Inc., pp. 101–199. https://doi.org/10.1016/bs.aivir.2014.11.006

Revers, F., García, J.A., 2015b. Molecular Biology of Potyviruses. Adv. Virus Res. 92, 101–199. https://doi.org/10.1016/BS.AIVIR.2014.11.006

Reyero-Saavedra, M. del R., Qiao, Z., Sánchez-Correa, M. del S., Reyes, J.L., Covarrubias, A.A., Libault, M., Valdés-López, O., 2017. Gene Silencing of Argonaute5 Negatively Affects the Establishment of the Legume-Rhizobia Symbiosis. Genes (Basel). 5, 1–19. https://doi.org/10.3390/genes8120352

Rivas, F. V, Tolia, N.H., Song, J.-J., Aragon, J.P., Liu, J., Hannon, G.J., Joshua-Tor, L., 2005. Purified Argonaute2 and an siRNA form recombinant human RISC. Nat. Struct. Mol. Biol. 12, 340–349. https://doi.org/10.1038/nsmb918

Rodríguez-Leal, D., Castillo-Cobián, A., Rodríguez-Arévalo, I., Vielle-Calzada, J.-P., 2016. A Primary Sequence Analysis of the ARGONAUTE Protein Family in Plants. Front. Plant Sci. 7, 1347. https://doi.org/10.3389/fpls.2016.01347

Roossinck, M.J., 2002. Evolutionary history of Cucumber mosaic virus deduced by phylogenetic analyses. J. Virol. 76, 3382–7. https://doi.org/10.1128/jvi.76.7.3382-3387.2002

Roossinck, M.J., 2001. Cucumber mosaic virus, a model for RNA virus evolution. Mol. Plant Pathol. 2, 59–63. https://doi.org/10.1046/j.1364-3703.2001.00058.x

Rosa, C., Kuo, Y.-W., Wuriyanghan, H., Falk, B.W., 2018. RNA Interference Mechanisms and Applications in Plant Pathology. Annu. Rev. Phytopathol. 56, 581–610. https://doi.org/10.1146/annurev-phyto-080417

Rose, L., Atwell, S., Grant, M., Holub, E.B., 2012. Parallel Loss-of-Function at the RPM1 Bacterial Resistance Locus in Arabidopsis thaliana. Front. Plant Sci. 3, 287. https://doi.org/10.3389/fpls.2012.00287

Roth, B.M., Pruss, G.J., Vance, V.B., 2004. Review-Plant viral suppressors of RNA silencing. Virus Res. 102, 97–108. https://doi.org/10.1016/j.virusres.2004.01.020

Sanjuán, R., Nebot, M.R., Chirico, N., Louis, M., Belshaw, R., Sanjua, R., Mansky, L.M., 2010.

Viral Mutation Rates Viral Mutation Rates □. J. Virol. 84, 9733–9748. https://doi.org/10.1128/JVI.00694-10

Saumet, A., Lecellier, C.-H., 2006. Anti-viral RNA silencing: do we look like plants? Retrovirology 3, 3. https://doi.org/10.1186/1742-4690-3-3

Schepetilnikov, M. V., Manske, U., Solovyev, A.G., Jr, A.A.Z., Schiemann, J., Morozov, S.Y., 2005. The hydrophobic segment of Potato virus X TGBp3 is a major determinant of the protein intracellular trafficking. J. Gen. Virol. 86, 2379–2391. https://doi.org/10.1099/vir.0.80865-0

Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Schölkopf, B., Weigel, D., Lohmann, J.U., 2005. A gene expression map of Arabidopsis thaliana development. Nat. Genet. 37, 501–506. https://doi.org/10.1038/ng1543

Scholthof, H.B., Alvarado, V.Y., Vega-Arreguin, J.C., Ciomperlik, J., Odokonyero, D., Brosseau, C., Jaubert, M., Zamora, A., Moffett, P., 2011. Identification of an ARGONAUTE for antiviral RNA silencing in Nicotiana benthamiana. Plant Physiol. 156, 1548–55. https://doi.org/10.1104/pp.111.178764

Schott, G., Mari-Ordonez, A., Himber, C., Alioua, A., Voinnet, O., Dunoyer, P., 2012. Differential effects of viral silencing suppressors on siRNA and miRNA loading support the existence of two distinct cellular pools of ARGONAUTE1. EMBO J. 31, 2553–65. https://doi.org/10.1038/emboj.2012.92

Schuck, J., Gursinsky, T., Pantaleo, V., Burgy??n, J., Behrens, S.E., 2013a. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic Acids Res. 41, 5090–5103.

Schuck, J., Gursinsky, T., Pantaleo, V., Burgyán, J., Behrens, S.-E., 2013b. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic Acids Res. 41, 5090–103. https://doi.org/10.1093/nar/gkt193

Senshu, H., Ozeki, J., Komatsu, K., Hashimoto, M., Hatada, K., Aoyama, M., Kagiwada, S., Yamaji, Y., Namba, S., 2017. Variability in the level of RNA silencing suppression caused

by triple gene block protein 1 (TGBp1) from various potexviruses during infection. https://doi.org/10.1099/vir.0.008243-0

Senshu, H., Ozeki, J., Komatsu, K., Hashimoto, M., Hatada, K., Aoyama, M., Kagiwada, S., Yamaji, Y., Namba, S., 2009. Variability in the level of RNA silencing suppression caused by triple gene block protein 1 (TGBp1) from various potexviruses during infection. J. Gen. Virol. https://doi.org/10.1099/vir.0.008243-0

Senthil-Kumar, M., Mysore, K.S., 2013. Nonhost Resistance Against Bacterial Pathogens: Retrospectives and Prospects. Annu. Rev. Phytopathol. https://doi.org/10.1146/annurev-phyto-082712-102319

Shamandi, N., Zytnicki, M., Charbonnel, C., Elvira-Matelot, E., Bochnakian, A., Comella, P., Mallory, A.C., Lepère, G., Sáez-Vásquez, J., Vaucheret, H., 2015a. Plants Encode a General siRNA Suppressor That Is Induced and Suppressed by Viruses. PLoS Biol. 13.

Shamandi, N., Zytnicki, M., Charbonnel, C., Elvira-Matelot, E., Bochnakian, A., Comella, P., Mallory, A.C., Lepère, G., Sáez-Vásquez, J., Vaucheret, H., 2015b. Plants Encode a General siRNA Suppressor That Is Induced and Suppressed by Viruses. PLOS Biol. 13, e1002326. https://doi.org/10.1371/journal.pbio.1002326

Shen, Q., Hu, T., Bao, M., Cao, L., Zhang, H., Song, F., Xie, Q., Zhou, X., 2016. Tobacco RING E3 Ligase NtRFP1 Mediates Ubiquitination and Proteasomal Degradation of a Geminivirus-Encoded βC1. Mol. Plant 9, 911–925. https://doi.org/10.1016/j.molp.2016.03.008

Shukla, A., Pagán, I., García-Arenal, F., 2018. Effective tolerance based on resource reallocation is a virus-specific defence in *Arabidopsis thaliana*. Mol. Plant Pathol. 19, 1454–1465. https://doi.org/10.1111/mpp.12629

Silhavy, D., Molnár, A., Lucioli, A., Szittya, G., Hornyik, C., Tavazza, M., Burgyán, J., 2002. A viral protein suppresses RNA silencing and binds silencing-generated, 21- to 25-nucleotide double-stranded RNAs. EMBO J. 21, 3070–80. https://doi.org/10.1093/emboj/cdf312

Siomi, M.C., Mannen, T., Siomi, H., 2010. How does the royal family of tudor rule the PIWI-interacting RNA pathway? Genes Dev. 24, 636–646. https://doi.org/10.1101/gad.1899210

Solovyev, A.G., Kalinina, N.O., Morozov, S.Y., 2012. Recent Advances in Research of Plant Virus Movement Mediated by Triple Gene Block. Front. Plant Sci. https://doi.org/10.3389/fpls.2012.00276

Solovyev, A.G., Stroganova, T.A., Zamyatnin, A.A., Fedorkin, O.N., Schiemann, J., Morozov, S.Y., 2000. Subcellular Sorting of Small Membrane-Associated Triple Gene Block Proteins: TGBp3-Assisted Targeting of TGBp2. Virology 269, 113–127. https://doi.org/10.1006/VIRO.2000.0200

Song, J.-J., Smith, S.K., HannonL, G.J., Joshua-Tor, L., 2004. Crystal Structure of Argonaute and Its Implications for RISC Slicer Activity. Science (80-.). 305, 1434–1437. https://doi.org/10.1126/science.1102514

Song, M.-S., Rossi, J.J., 2017. Molecular mechanisms of Dicer: endonuclease and enzymatic activity. Biochem. J. 474, 1603–1618. https://doi.org/10.1042/bcj20160759

Stael, S., Kmiecik, P., Willems, P., Van Der Kelen, K., Coll, N.S., Teige, M., Van Breusegem, F., 2015. Plant innate immunity--sunny side up? Trends Plant Sci. 20, 3–11. https://doi.org/10.1016/j.tplants.2014.10.002

Stahl, E.A., Dwyer, G., Mauricio, R., Kreitman, M., Bergelson, J., 1999. Dynamics of disease resistance polymorphism at the Rpm1 locus of Arabidopsis. Nature 400, 667–671. https://doi.org/10.1038/23260

Sun, Y., Sparks, C., Jones, H., Riley, M., Francis, F., Du, W., Xia, L., 2019. Silencing an essential gene involved in infestation and digestion in grain aphid through plant-mediated interference generates aphid-resistant wheat plants. Plant Biotechnol. J. 17, 852–854. https://doi.org/10.1111/pbi.13067

Syller, J., 2012. Facilitative and antagonistic interactions between plant viruses in mixed infections. Mol. Plant Pathol. https://doi.org/10.1111/j.1364-3703.2011.00734.x

Szittya, G., Burgyán, J., 2013. RNA Interference-Mediated Intrinsic Antiviral Immunity in Plants. https://doi.org/10.1007/978-3-642-37765-5_6

Szittya, G., Moxon, S., Pantaleo, V., Toth, G., Rusholme Pilcher, R.L., Moulton, V., Burgyan, J.,

Dalmay, T., 2010. Structural and functional analysis of viral siRNAs. PLoS Pathog. 6, e1000838. https://doi.org/10.1371/journal.ppat.1000838

Takahashi, H., Miller, J., Nozaki, Y., Takeda, M., Shah, J., Hase, S., Ikegami, M., Ehara, Y., Dinesh-Kumar, S.P., 2002. RCY1, an Arabidopsis thaliana RPP8/HRT family resistance gene, conferring resistance to cucumber mosaic virus requires salicylic acid, ethylene and a novel signal transduction mechanism. Plant J. 32, 655–667. https://doi.org/10.1046/j.1365-313X.2002.01453.x

Takahashi, H., Suzuki, M., Natsuaki, K., Shigyo, T., Hino, K., Teraoka, T., Hosokawa, D., Ehara, Y., 2001. Mapping the virus and host genes involved in the resistance response in cucumber mosaic virus-Infected Arabidopsis thaliana. Plant Cell Physiol. 42, 340–7.

Takeda, A., Iwasaki, S., Watanabe, T., Utsumi, M., Watanabe, Y., 2008. The Mechanism Selecting the Guide Strand from Small RNA Duplexes is Different Among Argonaute Proteins. Plant Cell Physiol. 49, 493–500. https://doi.org/10.1093/pcp/pcn043

Tecson Mendoza, E.M., C. Laurena, A., Botella, J.R., 2008. Recent advances in the development of transgenic papaya technology. Biotechnol. Annu. Rev. 14, 423–462. https://doi.org/10.1016/S1387-2656(08)00019-7

Thomas-Sharma, S., Abdurahman, A., Ali, S., Andrade-Piedra, J.L., Bao, S., Charkowski, A.O., Crook, D., Kadian, M., Kromann, P., Struik, P.C., Torrance, L., Garrett, K.A., Forbes, G.A., 2016. Seed degeneration in potato: the need for an integrated seed health strategy to mitigate the problem in developing countries. Plant Pathol. 65, 3–16. https://doi.org/10.1111/ppa.12439

Thompson, K., Stewart, A.J.A., 1981. The Measurement and Meaning of Reproductive Effort in Plants. Am. Nat. 117, 205–211. https://doi.org/10.1086/283700

Tilsner, J., Linnik, O., Wright, K.M., Bell, K., Roberts, A.G., Lacomme, C., Santa Cruz, S., Oparka, K.J., 2012. The TGB1 movement protein of Potato virus X reorganizes actin and endomembranes into the X-body, a viral replication factory. Plant Physiol. 158, 1359–70. https://doi.org/10.1104/pp.111.189605

Todesco, M., Balasubramanian, S., Hu, T.T., Traw, M.B., Horton, M., Epple, P., Kuhns, C., Sureshkumar, S., Schwartz, C., Lanz, C., Laitinen, R.A.E., Huang, Y., Chory, J., Lipka, V., Borevitz, J.O., Dangl, J.L., Bergelson, J., Nordborg, M., Weigel, D., 2010. Natural allelic variation underlying a major fitness trade-off in Arabidopsis thaliana. Nature 465. https://doi.org/10.1038/nature09083

Törjék, O., Meyer, R.C., Zehnsdorf, M., Teltow, M., Strompen, G., Witucka-Wall, H., Blacha, A., Altmann, T., 2008. Construction and Analysis of 2 Reciprocal Arabidopsis Introgression Line Populations. J. Hered. 99, 396–406. https://doi.org/10.1093/jhered/esn014

Tsuzuki, M., Takeda, A., Watanabe, Y., 2014. Recovery of dicer-like 1 -late flowering phenotype by miR172 expressed by the noncanonical DCL4-dependent biogenesis pathway. RNA 20, 1320–1327. https://doi.org/10.1261/rna.044966.114

Tucker, M.R., Okada, T., Hu, Y., Scholefield, a., Taylor, J.M., Koltunow, a. M.G., 2012. Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in Arabidopsis. Development 139, 1399–1404. https://doi.org/10.1242/dev.075390

Vagin, V. V, Wohlschlegel, J., Qu, J., Jonsson, Z., Huang, X., Chuma, S., Girard, A., Sachidanandam, R., Hannon, G.J., Aravin, A.A., 2009. methylation in specifying interaction with Tudor family members Proteomic analysis of murine Piwi proteins reveals a role for arginine methylation in specifying interaction with Tudor family members 1749–1762. https://doi.org/10.1101/gad.1814809

Vance, V., 2001. RNA Silencing in Plants--Defense and Counterdefense. Science (80-.). 292, 2277–2280. https://doi.org/10.1126/science.1061334

Várallyay, É., Oláh, E., Havelda, Z., 2014. Independent parallel functions of p19 plant viral suppressor of RNA silencing required for effective suppressor activity. Nucleic Acids Res. 42, 599–608. https://doi.org/10.1093/nar/gkt846

Varma, A. and M.N., 2001. Durable host-plant resistance, a desirable trait for integrated disease management.

Varsha Wesley, S., Helliwell, C.A., Smith, N.A., Wang, M., Rouse, D.T., Liu, Q., Gooding, P.S., Singh, S.P., Abbott, D., Stoutjesdijk, P.A., Robinson, S.P., Gleave, A.P., Green, A.G., Waterhouse, P.M., 2001. Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J. 27, 581–590. https://doi.org/10.1046/j.1365-313X.2001.01105.x

Vaucheret, H., 2008. Plant ARGONAUTES. Trends Plant Sci.

Vaucheret, H., Mallory, A.C., Bartel, D.P., 2006. AGO1 Homeostasis Entails Coexpression of MIR168 and AGO1 and Preferential Stabilization of miR168 by AGO1. Mol. Cell 22, 129–136.

Vaucheret, H., Vazquez, F., Crété, P., Bartel, D.P., 2004. The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. Genes Dev. 18, 1187–1197. https://doi.org/10.1101/gad.1201404

Verchot-Lubicz, J., Torrance, L., Solovyev, A.G., Morozov, S.Y., Jackson, A.O., Gilmer, D., 2010. Varied Movement Strategies Employed by Triple Gene Block–Encoding Viruses 23, 1231–1247. https://doi.org/10.1094

Verlaan, M.G., Hutton, S.F., Ibrahem, R.M., Kormelink, R., Visser, R.G.F., Scott, J.W., Edwards, J.D., Bai, Y., 2013. The Tomato Yellow Leaf Curl Virus Resistance Genes Ty-1 and Ty-3 Are Allelic and Code for DFDGD-Class RNA-Dependent RNA Polymerases. PLoS Genet. 9.

Vickers, T.A., Koo, S., Bennett, C.F., Crooke, S.T., Dean, N.M., Baker, B.F., 2003. Efficient reduction of target RNAs by small interfering RNA and RNase H-dependent antisense agents. A comparative analysis. J. Biol. Chem. 278, 7108–7118.

Voinnet, O., Lederer, C., Baulcombe, D.C., 2000. A Viral Movement Protein Prevents Spread of the Gene Silencing Signal in Nicotiana benthamiana. Cell 103, 157–167. https://doi.org/10.1016/S0092-8674(00)00095-7

Voinnet, O., Pinto, Y.M., Baulcombe, D.C., Lohuis, D., Visser, R.G.F., Bai, Y., Kormelink, R., 1999. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. Proc. Natl. Acad. Sci. U. S. A. 96, 14147–52.

https://doi.org/10.1073/pnas.96.24.14147

Wang, D., Liu, Q., Jones, H.D., Bruce, T., Xia, L., 2014. Comparative transcriptomic analyses revealed divergences of two agriculturally important aphid species. BMC Genomics 15. https://doi.org/10.1186/1471-2164-15-1023

Wang, J., Mei, J., Ren, G., 2019. Plant microRNAs: Biogenesis, Homeostasis, and Degradation. Front. Plant Sci. 10, 360. https://doi.org/10.3389/fpls.2019.00360

Wang, M.-B., Metzlaff, M., 2005. RNA silencing and antiviral defense in plants. Curr. Opin. Plant Biol. 8, 216–222. https://doi.org/10.1016/j.pbi.2005.01.006

Wang, X.-B., Jovel, J., Udomporn, P., Wang, Y., Wu, Q., Li, W.-X., Gasciolli, V., Vaucheret, H., Ding, S.-W., 2011. The 21-Nucleotide, but Not 22-Nucleotide, Viral Secondary Small Interfering RNAs Direct Potent Antiviral Defense by Two Cooperative Argonautes in Arabidopsis thaliana. Plant Cell 23, 1625–1638. https://doi.org/10.1105/tpc.110.082305

Wang, X.-B., Wu, Q., Ito, T., Cillo, F., Li, W.-X., Chen, X., Yu, J.-L., Ding, S.-W., 2010. RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 107, 484–489. https://doi.org/10.1073/pnas.0904086107

Wassenegger, M., Krczal, G., 2006. Nomenclature and functions of RNA-directed RNA polymerases. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2006.01.003

Wei, T., Wang, L., Zhou, X., Ren, X., Dai, X., Liu, H., 2015. PopW activates PAMP-triggered immunity in controlling tomato bacterial spot disease. Biochem. Biophys. Res. Commun. 463, 746–750.

Wei, W., Ba, Z., Gao, M., Wu, Y., Ma, Y., Amiard, S., White, C.I., Rendtlew Danielsen, J.M., Yang, Y.-G., Qi, Y., 2012. A Role for Small RNAs in DNA Double-Strand Break Repair. Cell 149, 101–112. https://doi.org/10.1016/j.cell.2012.03.002

Willmann, M.R., Endres, M.W., Cook, R.T., Gregory, B.D., 2011. The Functions of RNA-Dependent RNA Polymerases in Arabidopsis. Arab. B. 9, e0146. https://doi.org/10.1199/tab.0146

Wu, J., Yang, Z., Wang, Y., Zheng, L., Ye, R., Ji, Y., Zhao, S., Ji, S., Liu, R., Xu, L., Zheng, H., Zhou, Y., Zhang, X., Cao, X., Xie, L., Wu, Z., Qi, Y., Li, Y., 2015. Viral-inducible Argonaute18 confers broad-spectrum virus resistance in rice by sequestering a host microRNA. Elife 4. https://doi.org/10.7554/eLife.05733

Xiao, Y., Wu, K., 2019. Recent progress on the interaction between insects and Bacillus thuringiensis crops. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2018.0316

Xie, Z., Johansen, L.K., Gustafson, A.M., Kasschau, K.D., Lellis, A.D., Zilberman, D., Jacobsen, S.E., Carrington, J.C., 2004. Genetic and functional diversification of small RNA pathways in plants. PLoS Biol. 2, E104. https://doi.org/10.1371/journal.pbio.0020104

Xu, D., Zhou, G., 2017. Characteristics of siRNAs derived from Southern rice black-streaked dwarf virus in infected rice and their potential role in host gene regulation. Virol. J. 14, 27. https://doi.org/10.1186/s12985-017-0699-3

Yamaji, Y., Maejima, K., Ozeki, J., Komatsu, K., Shiraishi, T., Okano, Y., Himeno, M., Sugawara, K., Neriya, Y., Minato, N., Miura, C., Hashimoto, M., Namba, S., 2012. Lectin-mediated resistance impairs plant virus infection at the cellular level. Plant Cell 24, 778–93. https://doi.org/10.1105/tpc.111.093658

Yang, L., Li, B., Zheng, X.Y., Li, J., Yang, M., Dong, X., He, G., An, C., Deng, X.W., 2015. Salicylic acid biosynthesis is enhanced and contributes to increased biotrophic pathogen resistance in Arabidopsis hybrids. Nat. Commun. 6, 1–11. https://doi.org/10.1038/ncomms8309

Ye, J., Yang, J., Sun, Y., Zhao, P., Gao, S., Jung, C., Qu, J., Fang, R., Chua, N.H., 2015. Geminivirus Activates ASYMMETRIC LEAVES 2 to Accelerate Cytoplasmic DCP2-Mediated mRNA Turnover and Weakens RNA Silencing in Arabidopsis. PLoS Pathog. 11.

Ye, R., Wang, W., Iki, T., Liu, C., Wu, Y., Ishikawa, M., Zhou, X., Qi, Y., 2012. Cytoplasmic Assembly and Selective Nuclear Import of Arabidopsis ARGONAUTE4/siRNA Complexes. Mol. Cell 46, 859–870. https://doi.org/10.1016/j.molcel.2012.04.013

Yoshikawa, M., 2013. Biogenesis of trans-acting siRNAs, endogenous secondary siRNAs in plants. Genes Genet. Syst. 88, 77–84.

Yoshikawa, M., Iki, T., Tsutsui, Y., Miyashita, K., Poethig, R.S., Habu, Y., Ishikawa, M., 2013. 3′ fragment of miR173-programmed RISC-cleaved RNA is protected from degradation in a complex with RISC and SGS3. PNAS 110, 4117–4122. https://doi.org/10.1073/pnas.1217050110

Yu, X.D., Liu, Z.C., Huang, S.L., Chen, Z.Q., Sun, Y.W., Duan, P.F., Ma, Y.Z., Xia, L.Q., 2016. RNAi-mediated plant protection against aphids. Pest Manag. Sci.

Zhang, G., Chen, M., Li, L., Xu, Z., Chen, X., Guo, J., Ma, Y., 2009. Overexpression of the soybean GmERF3 gene, an AP2/ERF type transcription factor for increased tolerances to salt, drought, and diseases in transgenic tobacco. J. Exp. Bot. 60, 3781–96. https://doi.org/10.1093/jxb/erp214

Zhang, H., Xia, R., Walbot, V., 2015. Evolution, functions, and mysteries of plant ARGONAUTE proteins. Curr. Opin. Plant Biol. 27, 84–90. https://doi.org/10.1016/J.PBI.2015.06.011

Zhang, W., Sun, X., Yuan, H., Araki, H., Wang, J., Tian, D., 2008. The pattern of insertion/deletion polymorphism in Arabidopsis thaliana. Mol. Genet. Genomics 280, 351–361. https://doi.org/10.1007/s00438-008-0370-1

Zhang, Xiaoming, Xia, J., Lii, Y., Barrera-Figueroa, B., Zhou, X., Gao, S., Lu, L., Niu, D., Liang, W., Chen, Z., Leung, C., Wong, T., Guo, J., Li, Y., Liu, R., Zhu, J.-K., Zhang, W., Jin, H., Zhang, H., 2012. Genome-wide analysis of plant nat-siRNAs reveals insights into their distribution, biogenesis and function. Genome Biol. 13, R20.

Zhang, X., Yuan, Y.-R., Pei, Y., Lin, S.-S., Tuschl, T., Patel, D.J., Chua, N.-H., 2006. Cucumber mosaic virus-encoded 2b suppressor inhibits Arabidopsis Argonaute1 cleavage activity to counter plant defense. Genes Dev. 20, 3255–68. https://doi.org/10.1101/gad.1495506

Zhang, Xiaofeng, Zhang, X., Singh, J., Li, D., Qu, F., 2012. Temperature-Dependent Survival of Turnip Crinkle Virus-Infected Arabidopsis Plants Relies on an RNA Silencing-Based

Defense That Requires DCL2, AGO2, and HEN1. J. Virol. 86, 6847–6854. https://doi.org/10.1128/JVI.00497-12

Zhang, X., Zhao, H., Gao, S., Wang, W.-C., Katiyar-Agarwal, S., Huang, H.-D., Raikhel, N., Jin, H., 2011. Arabidopsis Argonaute 2 Regulates Innate Immunity via miRNA393∗-Mediated Silencing of a Golgi-Localized SNARE Gene, MEMB12. Mol. Cell 42, 356–366. https://doi.org/10.1016/j.molcel.2011.04.010

Zhang, Xiuchun, Zhang, Xiaofeng, Singh, J., Li, D., Qu, F., 2012. Temperature-dependent survival of Turnip crinkle virus-infected arabidopsis plants relies on an RNA silencing-based defense that requires dcl2, AGO2, and HEN1. J. Virol. 86, 6847–54. https://doi.org/10.1128/JVI.00497-12

Zhang, Z., Liu, X., Guo, X., Wang, X.-J., Zhang, X., 2016. Arabidopsis AGO3 predominantly recruits 24-nt small RNAs to regulate epigenetic silencing. Nat. Plants 2, 16049. https://doi.org/10.1038/nplants.2016.49

Zhao, K., Zhao, H., Chen, Z., Feng, L., Ren, J., Cai, R., Xiang, Y., 2015. The Dicer-like, Argonaute and RNA-dependent RNA polymerase gene families in Populus trichocarpa: gene structure, gene expression, phylogenetic analysis and evolution. J. Genet. 94, 317–321. https://doi.org/10.1007/s12041-015-0508-y

Zhao, Y., Chang, X., Qi, D., Dong, L., Wang, G., Fan, S., Jiang, L., Cheng, Q., Chen, X., Han, D., Xu, P., Zhang, S., 2017. A Novel Soybean ERF Transcription Factor, GmERF113, Increases Resistance to Phytophthora sojae Infection in Soybean. Front. Plant Sci. 8, 299. https://doi.org/10.3389/fpls.2017.00299

Zheng, X., Fahlgren, N., Abbasi, A., Berry, J.C., Carrington, J.C., 2019a. Antiviral ARGONAUTEs Against Turnip Crinkle Virus Revealed by Image-Based Trait Analysis. Plant Physiol. 180, 1418–1435. https://doi.org/10.1104/pp.19.00121

Zheng, X., Fahlgren, N., Abbasi, A., Berry, J.C., Carrington, J.C., 2019b. Antiviral ARGONAUTEs Against Turnip Crinkle Virus Revealed by Image-Based Trait Analysis. Plant Physiol. 180, 1418–1435. https://doi.org/10.1104/pp.19.00121

Zheng, X., Zhu, J., Kapoor, A., Zhu, J.-K., Liu, J., Chen, X., Harmer, S., Zilberman, D., Pikaard, C., Watson, J., Rezaian, A., Dennis, E., Waterhouse, P., Martienssen, R., 2007. Role of Arabidopsis AGO6 in siRNA accumulation, DNA methylation and transcriptional gene silencing. EMBO J. 26, 1691–701. https://doi.org/10.1038/sj.emboj.7601603

Zhou, X.-F., Sun, J.-D., Zhuo, Z., Jie, L. V, Wei, X.-W., Rui, C., Xu, H.-W., 2012. The Feasibility Analysis of PVX and TRV Vectors as the VIGS Tool for Studying the Gene Function peer-review under responsibility of [name organizer]. Int. Conf. Med. Phys. Biomed. Eng. 33, 46–54. https://doi.org/10.1016/j.phpro.2012.05.029

Zhou, Y., Honda, M., Duan, L., Zhang Correspondence, X., Zhu, H., Zhang, Z., Guo, X., Li, T., Li, Z., Peng, X., Nakajima, K., Zhang, X., 2015. Spatiotemporal Sequestration of miR165/166 by Arabidopsis Argonaute10 Promotes Shoot Apical Meristem Maintenance. CellReports 10, 1819–1827. https://doi.org/10.1016/j.celrep.2015.02.047

Zhu, H., Hu, F., Wang, R., Zhou, X., Sze, S.-H., Liou, L.W., Barefoot, A., Dickman, M., Zhang, X., Qi, Y, Chen, S., Hannon, G., Qi, Y, Chen, X., Chen, X., Wu, Z., Qi, Yijun, Li, Y., 2011. Arabidopsis Argonaute10 Specifically Sequesters miR166/165 to Regulate Shoot Apical Meristem Development. Cell 145, 242–256. https://doi.org/10.1016/j.cell.2011.03.024

Zilberman, D., Cao, X., Jacobsen, S.E., 2003. ARGONAUTE4 control of locus-specific siRNA accumulation and DNA and histone methylation. Science 299, 716–9. https://doi.org/10.1126/science.1079695

Zust, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., Barchet, W., Diamond, M.S., Siddell, S.G., Ludewig, B., Thiel, V., 2011. Ribose 2'-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. Nat Immunol 12, 137–143.

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5.9 Contributions

C.B. and P.M. conceived and designed the experiments. C.B., A.A., C.R.L., Z.Z. and S.B. performed the experiments. C.B. and A.A. analyzed the data. C.B. and P.M. wrote the article.

5.10 Competing interests

The authors declare no competing interests.

5.11 Supplementary Figures

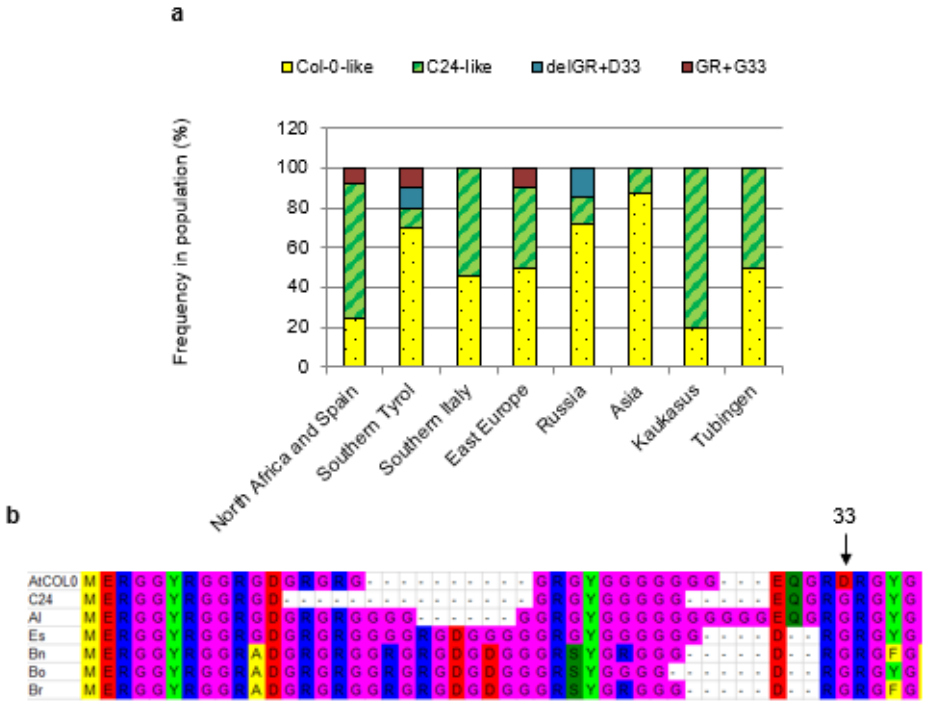


Figure S5.1. C24 and Col-0 alleles are found in all eight Eurasian populations analyzed. a, Chart representing allele frequencies in eight different Eurasian populations. Note that alleles have been classified based only on polymorphisms found in the GR motif and residue 33 of *AGO2* regardless of polymorphisms found elsewhere in the coding sequence. **b**, Amino acid alignment of a portion of the N-terminus of AGO2 from *Arabidopsis thaliana* Col-0 (AtCol-0), C24 (At-C24) and *Arabidopsis lyrata* (Al), as well as other *Brassicaceae*, *Eutrema salsugineum* (Es), *Brassica napus* (Bn), *Brassica oleracea* (Bo) and *Brassica rapa* (Br).


Central Asia
1. Col-0
2. C24
3. Sij-1
4. Sij-2
5. Sij-4
6. Sha
7. Koz-2
8. Leb-3
Russia
9. Shigu-1
10. Shigu-2
11. Stepn-2
12. Stepn-1
13. Borsk-2
14. Krazo-2
Kaukasus
15. Dog-4
16. Xan-1
17. Lerik1-3
18. Istisu-1
19. Yeg-1
20. Nemrut-1
East Europe
21. Dobra-1
22. Petro-1
23. Jablo-1
24. Bolin-1
25. Kastel-1
26. Koch-1
27. Del-10
28. Slavi-1

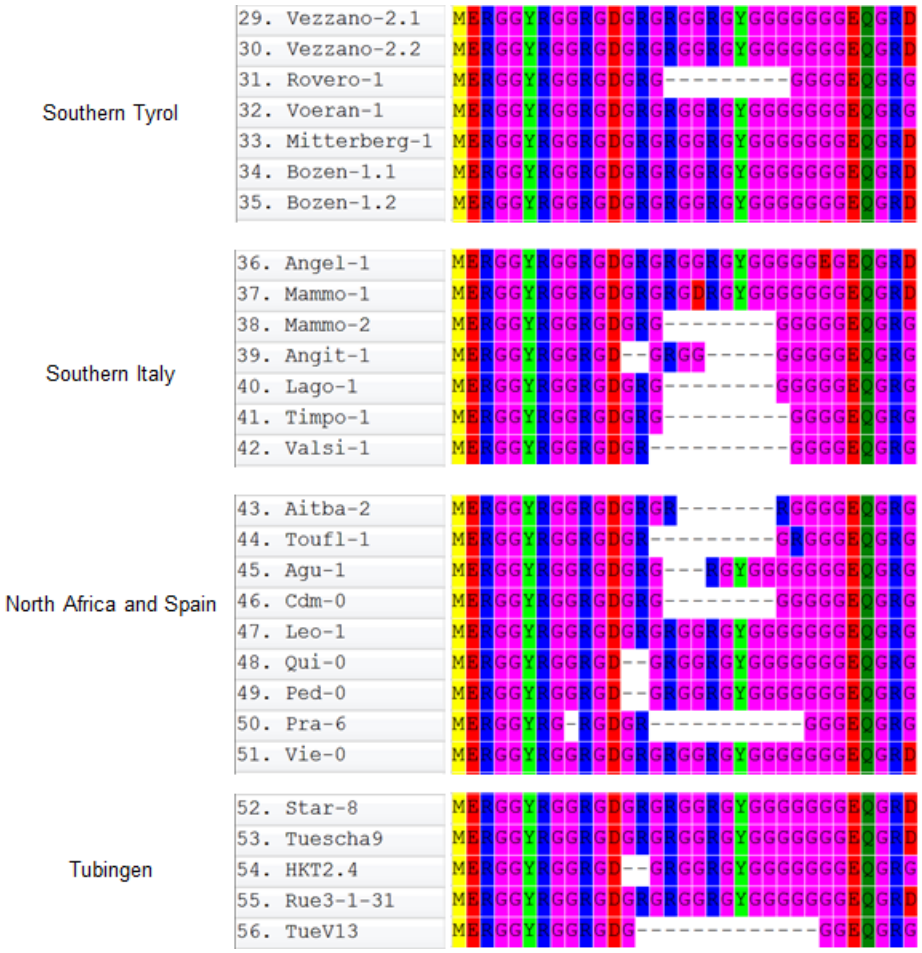


Figure S5.2. Alignment of the N-terminus of AGO2 of accessions tested in Figure 5.3. The first thirty-seven residue of Col-0 AGO2 were aligned using ClustalW with manual adjustment.

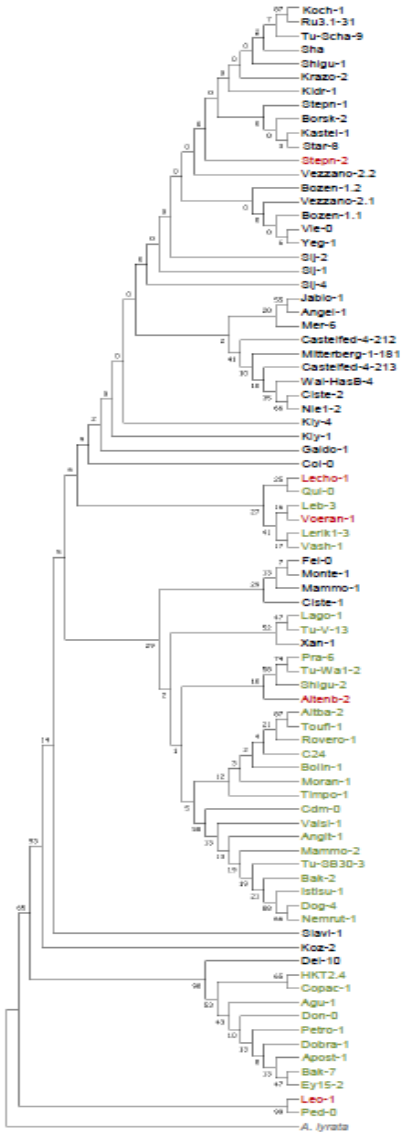
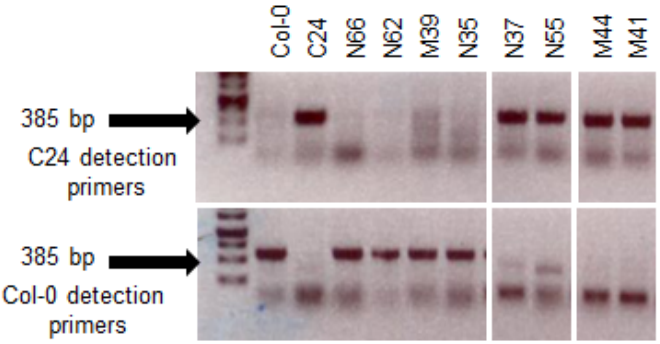


Figure S5.3. Phylogenetic analysis of Arabidopsis AGO2 sequences. Phylogenetic analysis was conducted on the entire Eurasian Arabidopsis accessions dataset and include Col-0 and C24 as well as *Arabidopsis lyrate AGO2* as an outgroup. Sequences aligned with ClustalW, using the following alignment parameters: for pairwise alignment, gap opening, 10.0, and gap extension, 0.1; for multiple alignment, gap opening, 10.0, and gap extension, 0.20. Resulting alignments were submitted to the Molecular Evolutionary Genetics Analysis 5 (MEGA5) software to generate a neighbor-joining tree derived from 5000 replicates. The evolutionary distances were computed using the JTT matrix-based method [3] and are in the units of the number of amino acid substitutions per site.

a



b

Background	Line	AGO2 allele	PVX in systemic leaves
Col-0	WT	Col-0	0/42
	ago2-1	none	33/42
	N35	Col-0	0/42
	N37	C24	21/42
	N55	C24	31/42
	N62	Col-0	2/42
	N66	Col-0	0/42
C24	WT	C24	40/42
	M39	Col-0	2/42
	M41	C24	40/42
	M44	C24	41/42

c

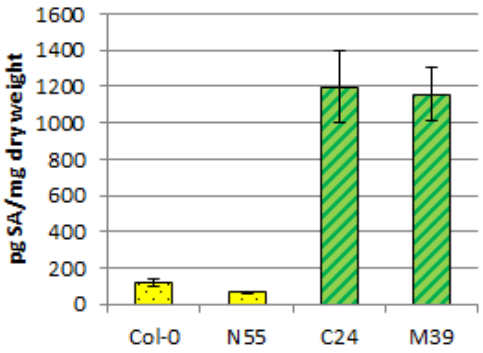


Figure S5.4. Correlation between *AGO2* alleles in RILS and susceptibility of *Arabidopsis* to PVX. a, Validation of *AGO2* alleles by PCR on gDNA of RILs with allele-specific primers. **b**, Numbers of PVX-inoculated plants showing systemic infection were scored by anti-PVX CP immunoblotting at 21 dpi. **c**, Quantification of SA content by LC-MS/MS in two different RILs and their respective background accessions, namely Col-0 and C24.

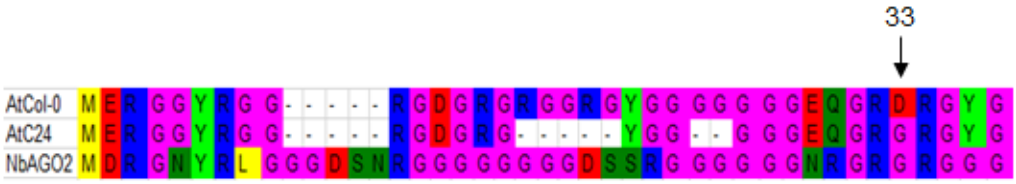


Figure S5.5. Alignment of the N-terminus of AGO2 proteins tested. The first thirty-seven residue of Col-0 AGO2 were aligned with, *Nicotiana benthamiana* (NbAGO2) and *Arabidopsis* C24 (C24) using ClustalW with manual adjustment.

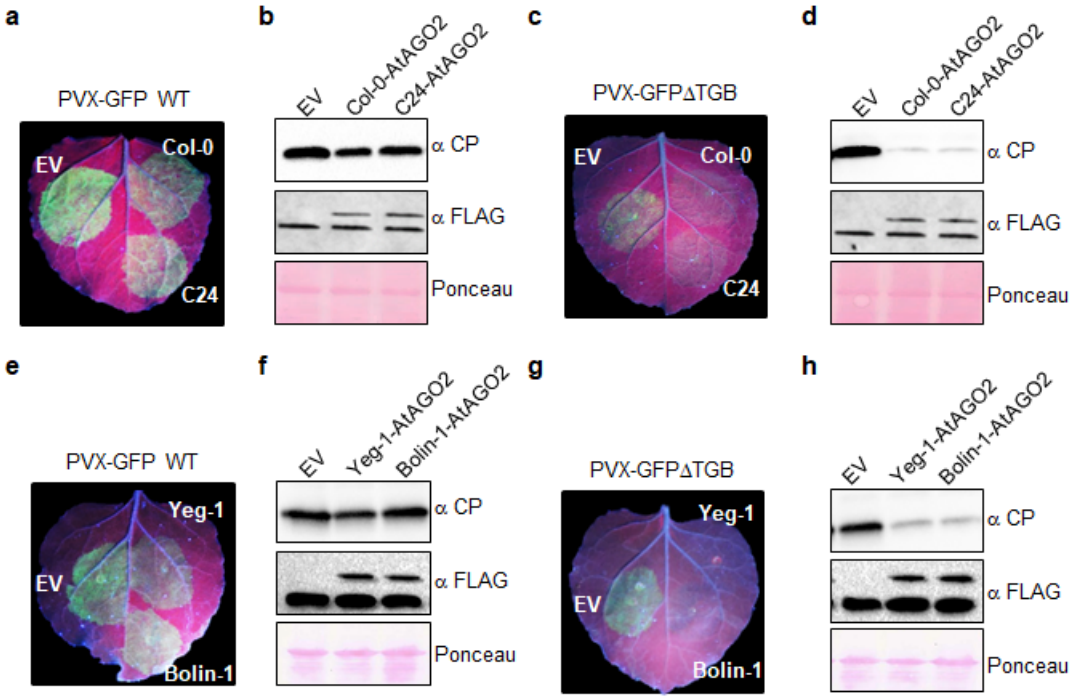


Figure S5.6. Polymorphisms found in C24 and C24-like *AGO2* affect its antiviral activity in *N. benthamiana*. a, c, e, g, *N. benthamiana* leaves were agroinfiltrated with PVX-GFP WT **a** and **c** or ΔTGB **e** and **g,** along with 35S:FLAG-Col-0-AGO2, 35S:FLAG-C24-AGO2 or empty vector (EV) **a** and **c** or with 35S:FLAG-AtYeg-1-AGO2, 35S:FLAG-AtBolin-1-AGO2 or empty vector (EV) **e** and **g**. Leaves were photographed under UV illumination at 4 dpi. **b**, **d**, **f, h**, Total protein extracts were prepared from *N. benthamiana* leaves agroinfiltrated as in **a**, **c**, **e**, **g** at 4 dpi and subjected to SDS-PAGE, followed by anti-PVX CP (top panel) or anti-FLAG (middle panel) immune blotting. Ponceau staining (bottom panel) of the same extracts is shown to demonstrate equal loading.

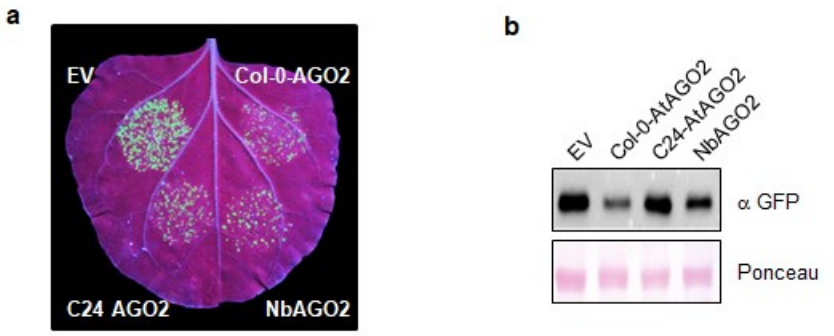


Figure S5.7. The difference in antiviral activity observed between the different alleles is also observed against PlAMV. a, *N. benthamiana* leaves were agroinfiltrated with PlAMV-GFP along with 35S:FLAG-AtCol-0-AGO2, 35S:FLAG-AtC24-AGO2, 35S:HA-NbAGO2 or empty vector (EV). Leaves were photographed under UV illumination at 4 days post infiltration (dpi). **b**, Total protein extracts were prepared from *N. benthamiana* leaves agroinfiltrated as in **a** at 4 dpi and subjected to SDS-PAGE, followed by anti-GFP immunoblotting (top panel). Ponceau staining (bottom panel) of the same extracts is shown to demonstrate equal loading.

BIBLIOGRAPHY

Adams, M.J., Antoniw, J.F., Fauquet, C.M., 2005. Molecular criteria for genus and species discrimination within the family Potyviridae. Arch. Virol. 150, 459–479. https://doi.org/10.1007/s00705-004-0440-6

Adenot, X., Elmayan, T., Lauressergues, D., Boutet, S., Bouché, N., Gasciolli, V., Vaucheret, H., 2006. DRB4-Dependent TAS3 trans-Acting siRNAs Control Leaf Morphology through AGO7. Curr. Biol. 16, 927–932. https://doi.org/10.1016/j.cub.2006.03.035

Agorio, A., Vera, P., 2007. ARGONAUTE4 is required for resistance to Pseudomonas syringae in Arabidopsis. Plant Cell 19, 3778–90. https://doi.org/10.1105/tpc.107.054494

Akbergenov, R., Si-Ammour, A., Blevins, T., Amin, I., Kutter, C., Vanderschuren, H., Zhang, P., Gruissem, W., Meins, F., Hohn, T., Pooggin, M.M., 2006. Molecular characterization of geminivirus-derived small RNAs in different plant species. Nucleic Acids Res. 34, 462–

471. https://doi.org/10.1093/nar/gkj447

Alazem, M., He, M.-H., Moffett, P., Lin, N.-S., 2017. Abscisic Acid Induces Resistance against Bamboo Mosaic Virus through Argonaute2 and 3. Plant Physiol. 174, 339–355. https://doi.org/10.1104/pp.16.00015

Anandalakshmi, R., Marathe, R., Ge, X., Herr, J.M., Mau, C., Mallory, A., Pruss, G., Bowman, L., Vance, V.B., 2000. A Calmodulin-Related Protein That Suppresses Posttranscriptional Gene Silencing in Plants. Science (80-.). 290.

Andika, I.B., Maruyama, K., Sun, L., Kondo, H., Tamada, T., Suzuki, N., 2015. Differential contributions of plant Dicer-like proteins to antiviral defences against potato virus X in leaves and roots. Plant J. 81, 781–793. https://doi.org/10.1111/tpj.12770

Ange´lique Deleris, Javier Gallego-Bartolome, Jinsong Bao, Kristin D. Kasschau, James C. Carrington, O.V., 2006. Hierarchical Action and Inhibition of Plant Dicer-Like Proteins in Antiviral Defense. Science (80-.). 305, 1462–322. https://doi.org/10.1126/science.1128650

Axtell, M.J., 2013. Classification and Comparison of Small RNAs from Plants. Annu. Rev. Plant Biol. 64, 137–159. https://doi.org/10.1146/annurev-arplant-050312-120043

Azevedo, J., Garcia, D., Pontier, D., Ohnesorge, S., Yu, A., Garcia, S., Braun, L., Bergdoll, M., Hakimi, M.A., Lagrange, T., Voinnet, O., 2010. Argonaute quenching and global changes in Dicer homeostasis caused by a pathogen-encoded GW repeat protein. Genes Dev. 24, 904–915. https://doi.org/10.1101/gad.1908710

Baumberger, N., Baulcombe, D.C., 2005. Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. Proc. Natl. Acad. Sci. U. S. A. 102, 11928–33.

Bejerman, N., Mann, K.S., Dietzgen, R.G., 2016. Alfalfa dwarf cytorhabdovirus P protein is a local and systemic RNA silencing supressor which inhibits programmed RISC activity and prevents transitive amplification of RNA silencing. Virus Res. 224, 19–28. https://doi.org/10.1016/j.virusres.2016.08.008

Bellaoui, M., Keddie, J.S., Gruissem, W., 2003. DCL is a plant-specific protein required for plastid ribosomal RNA processing and embryo development. Plant Mol. Biol. 53, 531–543.

Blevins, T., Rajeswaran, R., Shivaprasad, P. V., Beknazariants, D., Si-Ammour, A., Park, H.S., Vazquez, F., Robertson, D., Meins, F., Hohn, T., Pooggin, M.M., 2006. Four plant Dicers mediate viral small RNA biogenesis and DNA virus induced silencing. Nucleic Acids Res. 34, 6233–6246.

Bohmert, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M., Benning, C., 1998. AGO1 defines a novel locus of Arabidopsis controlling leaf development. EMBO J. 17, 170–180.

Bologna, N.G., Voinnet, O., 2014. The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis. Annu. Rev. Plant Biol. 65. https://doi.org/10.1146/annurev-arplant-050213-035728

Borges, F., Martienssen, R.A., 2015. The expanding world of small RNAs in plants. Nat. Publ. Gr. 16. https://doi.org/10.1038/nrm4085

Bortolamiol, D., Pazhouhandeh, M., Marrocco, K., Genschik, P., Ronique Ziegler-Graff, V., 2007. Report The Polerovirus F Box Protein P0 Targets ARGONAUTE1 to Suppress RNA Silencing. Curr. Biol. 17, 1615–1621. https://doi.org/10.1016/j.cub.2007.07.061

Bouché, N., Lauressergues, D., Gasciolli, V., Vaucheret, H., 2006. An antagonistic function for Arabidopsis DCL2 in development and a new function for DCL4 in generating viral siRNAs. EMBO J. 25, 3347–3356. https://doi.org/10.1038/sj.emboj.7601217

Boutrot, F., Zipfel, C., 2017. Function, Discovery, and Exploitation of Plant Pattern Recognition Receptors for Broad-Spectrum Disease Resistance. Annu. Rev. Phytopathol. 55, 257–286. https://doi.org/10.1146/annurev-phyto-080614-120106

Brodersen, P., Sakvarelidze-Achard, L., Bruun-Rasmussen, M., Dunoyer, P., Yamamoto, Y.Y., Sieburth, L., Voinnet, O., 2008. Widespread Translational Inhibition by Plant miRNAs and siRNAs. Science (80-.). 320, 1185–1190. https://doi.org/10.1126/science.1159151

Brosseau, C., Moffett, P., 2015. Functional and Genetic Analysis Identify a Role for Arabidopsis ARGONAUTE5 in Antiviral RNA Silencing. Plant Cell 27, 1742–54. https://doi.org/10.1105/tpc.15.00264

Brosseau, C., Oirdi, M. El, Adurogbangba, A., Ma, X., Moffett, P., 2016. Antiviral Defense Involves AGO4 in an Arabidopsis–Potexvirus Interaction. MPMI 29, 878–888.

https://doi.org/10.1094/MPMI-09-16-0188-R

Buchmann, R.C., Asad, S., Wolf, J.N., Mohannath, G., Bisaro, D.M., 2009. Geminivirus AL2 and L2 Proteins Suppress Transcriptional Gene Silencing and Cause Genome-Wide Reductions in Cytosine Methylation □. J. Virol. 83, 5005–5013. https://doi.org/10.1128/JVI.01771-08

Carbonell, A., Carrington, J.C., 2015. Antiviral roles of plant ARGONAUTES. Curr. Opin. Plant Biol. 27, 111–117. https://doi.org/10.1016/j.pbi.2015.06.013

Carbonell, A., Fahlgren, N., Garcia-Ruiz, H., Gilbert, K.B., Montgomery, T.A., Nguyen, T., Cuperus, J.T., Carrington, J.C., 2012. Functional analysis of three Arabidopsis ARGONAUTES using slicer-defective mutants. Plant Cell 24, 3613–29. https://doi.org/10.1105/tpc.112.099945

Carthew, R.W., Sontheimer, E.J., 2009. Origins and Mechanisms of miRNAs and siRNAs. Cell 136, 642–55. https://doi.org/10.1016/j.cell.2009.01.035

Chellappan, P., Vanitharani, R., Ogbe, F., Fauquet, C.M., 2005. Effect of temperature on geminivirus-induced RNA silencing in plants. Plant Physiol. 138, 1828–41. https://doi.org/10.1104/pp.105.066563

Chen, H., Zhang, L., Yu, K., Wang, A., 2015. Pathogenesis of Soybean mosaic virus in soybean carrying Rsv1 gene is associated with miRNA and siRNA pathways, and breakdown of AGO1 homeostasis. Virology 476, 395–404. https://doi.org/10.1016/j.virol.2014.12.034

Cheng, X., Wang, A., 2017. The Potyvirus Silencing Suppressor Protein VPg Mediates Degradation of SGS3 via Ubiquitination and Autophagy Pathways. J. Virol. 91. https://doi.org/10.1128/jvi.01478-16

Chisholm, S.T., Coaker, G., Day, B., Staskawicz, B.J., 2006. Host-microbe interactions: Shaping the evolution of the plant immune response. Cell.

Chiu, M.-H., Chen, I.-H., Baulcombe, D.C., Tsai, C.-H., 2010. The silencing suppressor P25 of Potato virus X interacts with Argonaute1 and mediates its degradation through the proteasome pathway. Mol. Plant Pathol. 11, 641–9. https://doi.org/10.1111/j.1364-3703.2010.00634.x

Cho, Y.B., Jones, S.I., Vodkin, L.O., 2017. Mutations in Argonaute5 Illuminate Epistatic Interactions of the K1 and I Loci Leading to Saddle Seed Color Patterns in Glycine max. Plant Cell 29, tpc.00162.2017. https://doi.org/10.1105/tpc.17.00162

Cook, D.E., Mesarich, C.H., Thomma, B.P.H.J., 2015. Understanding Plant Immunity as a Surveillance System to Detect Invasion. Annu. Rev. Phytopathol 53, 541–63. https://doi.org/10.1146/annurev-phyto-080614-120114

Csorba, T., Kontra, L., Burgyán, J., 2015. Viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. Virology. https://doi.org/10.1016/j.virol.2015.02.028

Csorba, T., Lózsa, R., Hutvágner, G., Burgyán, J., 2010. Polerovirus protein P0 prevents the assembly of small RNA-containing RISC complexes and leads to degradation of ARGONAUTE1. Plant J. 62, 463–472. https://doi.org/10.1111/j.1365-313X.2010.04163.x

Curtin, S.J., Michno, J.-M., Campbell, B.W., Gil-Humanes, J., Mathioni, S.M., Hammond, R., Gutierrez-Gonzalez, J.J., Donohue, R.C., Kantar, M.B., Eamens, A.L., Meyers, B.C., Voytas, D.F., Stupar, R.M., 2016. MicroRNA Maturation and MicroRNA Target Gene Expression Regulation Are Severely Disrupted in Soybean dicer-like1 Double Mutants. Genes, Genomes, Genet. 6, 423–433.

Dempsey, D.A., Klessig, D.F., 2012. SOS - too many signals for systemic acquired resistance? Trends Plant Sci.

Derrien, B., Baumberger, N., Schepetilnikov, M., Viotti, C., De Cillia, J., Ziegler-Graff, V., Isono, E., Schumacher, K., Genschik, P., 2012. Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. Proc. Natl. Acad. Sci. 109, 15942–15946. https://doi.org/10.1073/pnas.1209487109

Di Serio, F., Schö, H., Iglesias, A., Tarina, C., Bouldoires, E., Meins, F., Phillips, R.L., 2001. Sense-and antisense-mediated gene silencing in tobacco is inhibited by the same viral suppressors and is associated with accumulation of small RNAs. PNAS 98, 6506–6510.

Diao, P., Zhang, Q., Sun, H., Ma, W., Cao, A., Yu, R., Wang, J., Niu, Y., Wuriyanghan, H., Diao, P., Zhang, Q., Sun, H., Ma, W., Cao, A., Yu, R., Wang, J., Niu, Y., Wuriyanghan, H., 2019. miR403a and SA Are Involved in NbAGO2 Mediated Antiviral Defenses Against

TMV Infection in Nicotiana benthamiana. Genes (Basel). 10, 526. https://doi.org/10.3390/genes10070526

Donaire, L., Wang, Y., Gonzalez-Ibeas, D., Mayer, K.F., Aranda, M.A., Llave, C., 2009. Deep-sequencing of plant viral small RNAs reveals effective and widespread targeting of viral genomes. Virology 392, 203–214. https://doi.org/10.1016/j.virol.2009.07.005

Duan, C.-G., Fang, Y.-Y., Zhou, B.-J., Zhao, J.-H., Hou, W.-N., Zhu, H., Ding, S.-W., Guo, H.-S., 2012. Suppression of Arabidopsis ARGONAUTE1-Mediated Slicing, Transgene-Induced RNA Silencing, and DNA Methylation by Distinct Domains of the Cucumber mosaic virus 2b Protein. Plant Cell. https://doi.org/10.1105/tpc.111.092718

Duan, C.-G., Zhang, H., Tang, K., Zhu, X., Qian, W., Hou, Y.-J., Wang, B., Lang, Z., Zhao, Y., Wang, X., Wang, P., Zhou, J., Liang, G., Liu, N., Wang, C., Zhu, J.-K., 2015. Specific but interdependent functions for Arabidopsis AGO4 and AGO6 in RNA-directed DNA methylation. EMBO J. 34, 581–592. https://doi.org/10.15252/embj

Dzianott, A., Sztuba-Solińska, J., Bujarski, J.J., 2012. Mutations in the Antiviral RNAi Defense Pathway Modify Brome mosaic virus RNA Recombinant Profiles. Mol. Plant-Microbe Interact. 25, 97–106. https://doi.org/10.1094/MPMI-05-11-0137

El-Shami, M., Pontier, D., Lahmy, S., Braun, L., Picart, C., Vega, D., Hakimi, M.A., Jacobsen, S.E., Cooke, R., Lagrange, T., 2007. Reiterated WG/GW motifs form functionally and evolutionarily conserved ARGONAUTE-binding platforms in RNAi-related components. Genes Dev. 21, 2539–2544. https://doi.org/10.1101/gad.451207

Elsharkawy, M.M., Shimizu, M., Takahashi, H., Ozaki, K., Hyakumachi, M., 2013. Induction of Systemic Resistance against Cucumber mosaic virus in Arabidopsis thaliana by Trichoderma asperellum SKT-1. Plant Pathol. J. 29, 193. https://doi.org/10.5423/PPJ.SI.07.2012.01

Eun, C., Lorkovic, Z.J., Naumann, U., Long, Q., Havecker, E.R., Simon, S.A., Meyers, B.C., Matzke, A.J.M., Matzke, M., Freitag, M., 2011. AGO6 Functions in RNA-Mediated Transcriptional Gene Silencing in Shoot and Root Meristems in Arabidopsis thaliana. https://doi.org/10.1371/journal.pone.0025730

Fagard, M., Boutet, S., Morel, J.-B., Bellini, C., Vaucheret, H., 2000. AGO1, QDE-2, and RDE-1 are required for post-transcriptional gene silencing in plants , quelling in fungi , and RNA interference in animals. Proc. Natl. Acad. Sci. 97, 11650–11654.

Fang, X., Qi, Y., 2016. RNAi in Plants: An Argonaute-Centered View. Plant Cell 28, 272–85. https://doi.org/10.1105/tpc.15.00920

Fang, Y.-Y., Zhao, J.-H., Liu, S.-W., Wang, S., Duan, C.-G., Guo, H.-S., 2016. CMV2b-AGO Interaction Is Required for the Suppression of RDR-Dependent Antiviral Silencing in Arabidopsis. Front. Microbiol. 7, 1329. https://doi.org/10.3389/fmicb.2016.01329

Fátyol, K., Ludman, M., Burgyán, J., P.D., Z., V., S., T., T., D.J., P., H., V., W.M., K., R., C., S., C., G.J., H., Y., Q., 2016. Functional dissection of a plant Argonaute. Nucleic Acids Res. 44, 1384–1397. https://doi.org/10.1093/nar/gkv1371

Feng, L., Duan, C.-G., Guo, H.-S., 2013. Inhibition of *in vivo* Slicer activity of Argonaute protein 1 by the viral 2b protein independent of its dsRNA-binding function. Mol. Plant Pathol. 14, 617–622. https://doi.org/10.1111/mpp.12033

Fusaro, A.F., Matthew, L., Smith, N.A., Curtin, S.J., Dedic-Hagan, J., Ellacott, G.A., Watson, J.M., Wang, M.-B., Brosnan, C., Carroll, B.J., Waterhouse, P.M., 2006. RNA interference-inducing hairpin RNAs in plants act through the viral defence pathway. EMBO Rep. 7, 1168–75. https://doi.org/10.1038/sj.embor.7400837

Garcia-Ruiz, H., Carbonell, A., Hoyer, J.S., Fahlgren, N., Gilbert, K.B., Takeda, A., Giampetruzzi, A., Ruiz, M.T.G., Mcginn, M.G., Lowery, N., Martinez Baladejo, M.T., Carrington, J.C., -Ruiz, G., Carbonell, H., Js, H., Ding, B., 2015. Roles and Programming of Arabidopsis ARGONAUTE Proteins during Turnip Mosaic Virus Infection. PLoS Pathog 11. https://doi.org/10.1371/journal.ppat.1004755

Ghoshal, B., Sanfaçon, H., 2014. Temperature-dependent symptom recovery in Nicotiana benthamiana plants infected with tomato ringspot virus is associated with reduced translation of viral RNA2 and requires ARGONAUTE 1. Virology 456–457, 188–197. https://doi.org/10.1016/J.VIROL.2014.03.026

Giner, A., Lakatos, L., García-Chapa, M., López-Moya, J.J., Burgyán, J., 2010. Viral Protein

Inhibits RISC Activity by Argonaute Binding through Conserved WG/GW Motifs. PLoS Pathog. 6, e1000996. https://doi.org/10.1371/journal.ppat.1000996

Gonzalez-Gaitan, M., Jessell, T., Johnston, L., Laufer, E., McCabe, B., Struhl, G., Deleris, A., Gallego-Bartolome, J., Bao, J., Kasschau, K.D., Carrington, J.C., Voinnet, O., 2004. Hierarchical Action and Inhibition of Plant Dicer-Like Proteins in Antiviral Defense. Science (80-.). 305, 1462–322. https://doi.org/10.1126/science.1128650

González, I., Martínez, L., Rakitina, D. V., Lewsey, M.G., Atencio, F.A., Llave, C., Kalinina, N.O., Carr, J.P., Palukaitis, P., Canto, T., 2010. Cucumber mosaic virus 2b protein subcellular targets and interactions: Their significance to RNA silencing suppressor activity. Mol. Plant-Microbe Interact. 23, 294–303. https://doi.org/10.1094/MPMI-23-3-0294

González, I., Rakitina, D., Semashko, M., Taliansky, M., Praveen, S., Palukaitis, P., Carr, J.P., Kalinina, N., Canto, T., 2012. RNA binding is more critical to the suppression of silencing function of Cucumber mosaic virus 2b protein than nuclear localization. RNA 18, 771–782. https://doi.org/10.1261/rna.031260.111

Goto, K., Kobori, T., Kosaka, Y., Natsuaki, T., Masuta, C., 2007. Characterization of Silencing Suppressor 2b of Cucumber Mosaic Virus Based on Examination of its Small RNA-Binding Abilities. Plant Cell Physiol. 48, 1050–1060. https://doi.org/10.1093/pcp/pcm074

Ha, M., Kim, V.N., 2014. Regulation of microRNA biogenesis. Nat. Rev. Mol. Cell Biol. 15, 509–524.

Haas, G., Azevedo, J., Moissiard, G., Le Geldreich, A., Himber, C., Bureau, M., Fukuhara, T., Keller, M., Voinnet, O., 2008. Nuclear import of CaMV P6 is required for infection and suppression of the RNA silencing factor DRB4. EMBO J. 27129, 2102–2112.

Hamera, S., Song, X., Su, L., Chen, X., Fang, R., 2012. Cucumber mosaic virus suppressor 2b binds to AGO4-related small RNAs and impairs AGO4 activities. Plant J. 69, 104–115.

Harvey, J.J.W., Lewsey, M.G., Patel, K., Westwood, J., Heimstädt, S., Carr, J.P., Baulcombe, D.C., 2011. An antiviral defense role of AGO2 in plants. PLoS One 6.

Hauptmann, J., Schraivogel, D., Bruckmann, A., Manickavel, S., Jakob, L., Eichner, N., Pfaff, J., Urban, M., Sprunck, S., Hafner, M., Tuschl, T., Deutzmann, R., Meister, G., 2015.

Biochemical isolation of Argonaute protein complexes by Ago-APP. Proc. Natl. Acad. Sci. U. S. A. 112, 11841–5. https://doi.org/10.1073/pnas.1506116112

Havecker, E.R., Wallbridge, L.M., Hardcastle, T.J., Bush, M.S., Kelly, K.A., Dunn, R.M., Schwach, F., Doonan, J.H., Baulcombe, D.C., 2010. The Arabidopsis RNA-directed DNA methylation argonautes functionally diverge based on their expression and interaction with target loci. Plant Cell 22, 321–34. https://doi.org/10.1105/tpc.109.072199

Hernández-Lagana, E., Rodríguez-Leal, D., Lúa, J., Vielle-Calzada, J.P., 2016. A multigenic network of ARGONAUTE4 clade members controls early megaspore formation in arabidopsis. Genetics 204, 1045–1056. https://doi.org/10.1534/genetics.116.188151

Hily, J.M., García, A., Moreno, A., Plaza, M., Wilkinson, M.D., Fereres, A., Fraile, A., García-Arenal, F., 2014. The Relationship between Host Lifespan and Pathogen Reservoir Potential: An Analysis in the System Arabidopsis thaliana-Cucumber mosaic virus. PLoS Pathog. 10, e1004492. https://doi.org/10.1371/journal.ppat.1004492

Howell, M.D., Fahlgren, N., Chapman, E.J., Cumbie, J.S., Sullivan, C.M., Givan, S.A., Kasschau, K.D., Carrington, J.C., 2007. Genome-wide analysis of the RNA-DEPENDENT RNA POLYMERASE6/DICER-LIKE4 pathway in Arabidopsis reveals dependency on miRNA- and tasiRNA-directed targeting. Plant Cell 19, 926–42. https://doi.org/10.1105/tpc.107.050062

Hunter, C., 2006. Trans-acting siRNA-mediated repression of ETTIN and ARF4 regulates heteroblasty in Arabidopsis. Development 133, 2973–2981. https://doi.org/10.1242/dev.02491

Inoue-Nagata, A.K., Lima, M.F., Gilbertson, R.L., Inoue-Nagata, A.K., Lima, M.F., Gilbertson, R.L., 2016. A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. Hortic. Bras. 34, 8–18. https://doi.org/10.1590/S0102-053620160000100002

Ivanov, K.I., Eskelin, K., Bašić, M., De, S., Lõhmus, A., Varjosalo, M., Mäkinen, K., 2016. Molecular insights into the function of the viral RNA silencing suppressor HCPro. Plant J. 85, 30–45. https://doi.org/10.1111/tpj.13088

Ivanov, K.I., Eskelin, K., Lõ, A., Mä Kinen Correspondence, K., Mä, K., 2014. Molecular and cellular mechanisms underlying potyvirus infection. J. Gen. Virol. 95, 1415–1429. https://doi.org/10.1099/vir.0.064220-0

Iwasaki, S., Kobayashi, M., Yoda, M., Sakaguchi, Y., Katsuma, S., Suzuki, T., Tomari, Y., 2010. Hsc70/Hsp90 Chaperone Machinery Mediates ATP-Dependent RISC Loading of Small RNA Duplexes. Mol. Cell 39, 292–299. https://doi.org/10.1016/j.molcel.2010.05.015

Jacquemond, M., 2012. Cucumber Mosaic Virus. Adv. Virus Res. 84, 439–504. https://doi.org/10.1016/B978-0-12-394314-9.00013-0

Jamous, R.M., Boonrod, K., Fuellgrabe, M.W., Ali-Shtayeh, M.S., Krczal, G., Wassenegger, M., 2011. The helper component-proteinase of the Zucchini yellow mosaic virus inhibits the Hua Enhancer 1 methyltransferase activity in vitro. J. Gen. Virol. 92, 2222–2226.

Jaubert, M., Bhattacharjee, S., Mello, A.F.S., Perry, K.L., Moffett, P., 2011. ARGONAUTE2 Mediates RNA-Silencing Antiviral Defenses against Potato virus X in Arabidopsis 1[W][OA]. Plant Physiol. 156. https://doi.org/10.1104/pp.111.178012

Jenner, C.E., Wang, X., Ponz, F., Walsh, J.A., 2002. A fitness cost for Turnip mosaic virus to overcome host resistance. Virus Res. 86, 1–6. https://doi.org/10.1016/S0168-1702(02)00031-X

Jens Tilsner, O.L., Louveaux, M., Ian M. Roberts, Sean N. Chapman, and Karl J. Oparka, 2013. Replication and trafficking of a plant virus are coupled at the entrances of plasmodesmata. J. Cell Biol 201, 981–995. https://doi.org/10.1083/jcb.201304003

Jones, J.D.G., Dangl, J.L., 2006. The plant immune system. Nature 444, 323–329.

Jones, J.D.G., Vance, R.E., Dangl, J.L., 2016. Intracellular innate immune surveillance devices in plants and animals. Science (80-.). 354, 1117. https://doi.org/10.1126/science.aaf6395

Jouannet, V., Moreno, A.B., Elmayan, T., Vaucheret, H., Crespi, M.D., Maizel, A., 2012. Cytoplasmic Arabidopsis AGO7 accumulates in membrane-associated siRNA bodies and is required for ta-siRNA biogenesis. EMBO J. 31, 1704–1713. https://doi.org/10.1038/emboj.2012.20

Kapoor, M., Arora, R., Lama, T., Nijhawan, A., Khurana, J.P., Tyagi, A.K., Kapoor, S., 2008. Genome-wide identification, organization and phylogenetic analysis of Dicer-like, Argonaute and RNA-dependent RNA Polymerase gene families and their expression analysis during reproductive development and stress in rice. BMC Genomics 9. https://doi.org/10.1186/1471-2164-9-451

Kasschau, K.D., Carrington, J.C., 1998. A counterdefensive strategy of plant viruses: Suppression of posttranscriptional gene silencing. Cell 95, 461–470. https://doi.org/10.1016/S0092-8674(00)81614-1

Kidner, C.A., Martienssen, R.A., 2005. The role of ARGONAUTE1 (AGO1) in meristem formation and identity. Dev. Biol. 280, 504–517. https://doi.org/10.1016/J.YDBIO.2005.01.031

Kim, V.N., 2005. Small RNAs: classification, biogenesis, and function. Mol. Cells 19, 1–15. https://doi.org/806 [pii]

Kontra, L., Csorba, T., Tavazza, M., Lucioli, A., Tavazza, R., Moxon, S., Tisza, V., Medzihradszky, A., Turina, M., Burgyán, J., 2016. Distinct Effects of p19 RNA Silencing Suppressor on Small RNA Mediated Pathways in Plants. PLoS Pathog. 12, e1005935. https://doi.org/10.1371/journal.ppat.1005935

Kurihara, Y., Watanabe, Y., 2004. Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. PNAS 101, 12753–12758.

Lakatos, L., Szittya, G., Silhavy, D., Burgyán, J., 2004. Molecular mechanism of RNA silencing suppression mediated by p19 protein of tombusviruses. EMBO J. 23, 876–884.

Lario, L.D., Ramirez-Parra, E., Gutierrez, C., Spampinato, C.P., Casati, P., 2013. ANTI-SILENCING FUNCTION1 Proteins Are Involved in Ultraviolet-Induced DNA Damage Repair and Are Cell Cycle Regulated by E2F Transcription Factors in Arabidopsis 1[C][W][OA]. Plant Physiol. 162, 1164–1177. https://doi.org/10.1104/pp.112.212837

Laufs, J., Trautt, W., Heyraudt, F., Matzeitt, V., Rogersf, S.G., Schellt, J., Gronenborn, B., 1995. In vitro cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. Biochemistry 92, 3879–3883.

Laura R. Newburn, K.A.W., 2015. Cis-acting RNA elements in positive-strand RNA plant virus genomes. Virology 479, 434–443.

Lee, S.-C., Wu, C.-H., Wang, C.-W., 2010. Traffic of a Viral Movement Protein Complex to the Highly Curved Tubules of the Cortical Endoplasmic Reticulum. Traffic 11, 912–930. https://doi.org/10.1111/j.1600-0854.2010.01064.x

Li, C.F., Henderson, I.R., Song, L., Fedoroff, N., Lagrange, T., Jacobsen, S.E., 2008. Dynamic regulation of ARGONAUTE4 within multiple nuclear bodies in Arabidopsis thaliana. PLoS Genet. 4. https://doi.org/10.1371/journal.pgen.0040027

Li, C.F., Pontes, O., El-Shami, M., Henderson, I.R., Bernatavichute, Y. V., Chan, S.W.L., Lagrange, T., Pikaard, C.S., Jacobsen, S.E., 2006. An ARGONAUTE4-Containing Nuclear Processing Center Colocalized with Cajal Bodies in Arabidopsis thaliana. Cell 126, 93–106. https://doi.org/10.1016/j.cell.2006.05.032

Li, F., Wang, A., 2019. RNA-Targeted Antiviral Immunity: More Than Just RNA Silencing. Trends Microbiol. xx, 1–14. https://doi.org/10.1016/j.tim.2019.05.007

Li, F., Zhao, N., Li, Z., Xu, X., Wang, Y., Yang, X., Liu, S.-S., Wang, A., Zhou, X., 2017. A calmodulin-like protein suppresses RNA silencing and promotes geminivirus infection by degrading SGS3 via the autophagy pathway in Nicotiana benthamiana. PLoS Pathog. 13, e1006213. https://doi.org/10.1371/journal.ppat.1006213

Liang Wu, Huan Chen, C.C.& Z.Q.F., 2014. Go in for the kill: How plants deploy effectortriggered immunity to combat pathogens. Virulence 5, 710–721. https://doi.org/10.4161/viru.29755

Lico, C., Benvenuto, E., Baschieri, S., 2015. The Two-Faced Potato Virus X: From Plant Pathogen to Smart Nanoparticle. Front. Plant Sci. 6, 1009. https://doi.org/10.3389/fpls.2015.01009

Liu, J., Carmell, M. a, Rivas, F. V, Marsden, C.G., Thomson, J.M., Song, J.-J., Hammond, S.M., Joshua-Tor, L., Hannon, G.J., 2004. Argonaute2 is the catalytic engine of mammalian RNAi. Science 305, 1437–1441. https://doi.org/10.1126/science.1102513

Liu, Q., Feng, Y., Zhu, Z., 2009. Dicer-like (DCL) proteins in plants. Funct. Integr. Genomics.

Lough, T.J., Lee, R.H., Emerson, S.J., Forster, R.L.S., Lucas, W.J., 2006. Functional analysis of the 5′ untranslated region of potexvirus RNA reveals a role in viral replication and cell-to-cell movement. Virology 351, 455–465. https://doi.org/10.1016/J.VIROL.2006.03.043

Ludman, M., Burgyán, J., Fátyol, K., 2017. Crispr/Cas9 Mediated Inactivation of Argonaute 2 Reveals its Differential Involvement in Antiviral Responses. Sci. Rep. 7, 1010. https://doi.org/10.1038/s41598-017-01050-6

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P., Barton, M.K., 1999. The PINHEAD / ZWILLE gene acts pleiotropically in Arabidopsis development and has overlapping functions with the ARGONAUTE1 gene. Development 481, 469–481.

Ma, J.-B., Ye, K., Patel, D.J., 2004. Structural basis for overhang-specific small interfering RNA recognition by the PAZ domain. Nature 429, 318–22. https://doi.org/10.1038/nature02519

Ma, X., Nicole, M.-C., Meteignier, L.-V., Hong, N., Wang, G., Moffett, P., 2015. Different roles for RNA silencing and RNA processing components in virus recovery and virus-induced gene silencing in plants. J. Exp. Bot. 66, 919–932. https://doi.org/10.1093/jxb/eru447

Mallory, A., Vaucheret, H., 2010. Form, function, and regulation of ARGONAUTE proteins. Plant Cell 22, 3879–89. https://doi.org/10.1105/tpc.110.080671

Mallory, A.C., Hinze, A., Tucker, M.R., Bouche, N., Gasciolli, V., Elmayan, T., Lauressergues, D., Jauvion, V., Vaucheret, H., Laux, T., 2009. Redundant and Specific Roles of the ARGONAUTE Proteins AGO1 and ZLL in Development and Small RNA- Directed Gene Silencing. PLoS Genet. 5, 1–12. https://doi.org/10.1371/journal.pgen.1000646

Manavella, P.A., Koenig, D., Weigel, D., 2012. Plant secondary siRNA production determined by microRNA-duplex structure. Proc. Natl. Acad. Sci. U. S. A. 109, 2461–6.

Matzke, M. a, Kanno, T., Matzke, A.J.M., 2014. RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants. Annu. Rev. Plant Biol. 1–25. https://doi.org/10.1146/annurev-arplant-043014-114633

Matzke, M.A., Kanno, T., Matzke, A.J.M., 2015. RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants. Annu. Rev. Plant Biol. 66, 243–267. https://doi.org/10.1146/annurev-arplant-043014-114633

Maunoury, N., Vaucheret, H., 2011. AGO1 and AGO2 Act Redundantly in miR408-Mediated Plantacyanin Regulation. PLoS One 6, e28729. https://doi.org/10.1371/journal.pone.0028729

McCue, A.D., Panda, K., Nuthikattu, S., Choudury, S.G., Thomas, E.N., Slotkin, R.K., 2015. ARGONAUTE 6 bridges transposable element mRNA-derived siRNAs to the establishment of DNA methylation. EMBO J. 34, 20–35. https://doi.org/10.15252/embj.201489499

McHale, L., Tan, X., Koehl, P., Michelmore, R.W., 2006. Plant NBS-LRR proteins: adaptable guards. Genome Biol. 7, 212. https://doi.org/10.1186/gb-2006-7-4-212

Mérai, Z., Kerényi, Z., Molnár, A., Barta, E., Válóczi, A., Bisztray, G., Havelda, Z., Burgyán, J., Silhavy, D., 2005. Aureusvirus P14 is an efficient RNA silencing suppressor that binds double-stranded RNAs without size specificity. J. Virol. 79, 7217–26. https://doi.org/10.1128/JVI.79.11.7217-7226.2005

Mi, S., Cai, T., Hu, Y., Chen, Y., Hodges, E., Ni, F., Wu, L., Li, S., Zhou, H., Long, C., Chen, S., Hannon, G.J., Qi, Y., 2008. Sorting of Small RNAs into Arabidopsis Argonaute Complexes Is Directed by the 5′ Terminal Nucleotide. Cell 133, 116–127. https://doi.org/10.1016/j.cell.2008.02.034

Mingot, A., Valli, A., Rodamilans, B., San León, D., Baulcombe, D.C., García, J.A., López-Moya, J.J., 2016. The P1N-PISPO trans -Frame Gene of Sweet Potato Feathery Mottle Potyvirus Is Produced during Virus Infection and Functions as an RNA Silencing Suppressor . J. Virol. 90, 3543–3557. https://doi.org/10.1128/jvi.02360-15

Mlotshwa, S., Pruss, G.J., Vance, V., 2008. Small RNAs in viral infection and host defense. Trends Plant Sci. 13, 375–382. https://doi.org/10.1016/j.tplants.2008.04.009

Molnar, A., Melnyk, C.W., Bassett, A., Hardcastle, T.J., Dunn, R., Baulcombe, D.C., 2010. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science 328, 872–875. https://doi.org/10.1126/science.1187959

Montavon, T., Kwon, Y., Zimmermann, A., Hammann, P., Vincent, T., Cognat, V., Michel, F., Dunoyer, P., 2017. A specific dsRNA-binding protein complex selectively sequesters endogenous inverted-repeat siRNA precursors and inhibits their processing. Nucleic Acids

Res. 45, 1330–1344. https://doi.org/10.1093/nar/gkw1264

Montes, N., Alonso-Blanco, C., García-Arenal, F., 2019. Cucumber mosaic virus infection as a potential selective pressure on Arabidopsis thaliana populations. PLOS Pathog. 15, e1007810. https://doi.org/10.1371/journal.ppat.1007810

Montgomery, T.A., Howell, M.D., Cuperus, J.T., Li, D., Hansen, J.E., Alexander, A.L., Chapman, E.J., Fahlgren, N., Allen, E., Carrington, J.C., 2008. Specificity of ARGONAUTE7-miR390 Interaction and Dual Functionality in TAS3 Trans-Acting siRNA Formation. Cell 133, 128–141. https://doi.org/10.1016/j.cell.2008.02.033

Morel, J., Godon, C., Mourrain, P., Béclin, C., Boutet, S., Feuerbach, F., Proux, F., Vaucheret, H., 2002. Fertile Hypomorphic *ARGONAUTE* (*ago1*) mutants impaired in post-transcriptional gene silencing and virus resistance. Society 14, 629–639. https://doi.org/10.1105/tpc.010358.are

Moreno, A.B., Martínez De Alba, A.E., Bardou, F., Crespi, M.D., Vaucheret, H., Maizel, A., Mallory, A.C., 2013. Cytoplasmic and nuclear quality control and turnover of single-stranded RNA modulate post-transcriptional gene silencing in plants. Nucleic Acids Res. 41, 4699–4708. https://doi.org/10.1093/nar/gkt152

Mourrain, P., Bé, C., Elmayan, T., Feuerbach, F., Godon, C., Morel, J.-B., Jouette, D., Lacombe, A.-M., Nikic, S., Picault, N., Ré, K., Sanial, M., Vo, T.-A., Vaucheret, H., 2000. Arabidopsis SGS2 and SGS3 Genes Are Required for Posttranscriptional Gene Silencing and Natural Virus Resistance gene required for PTGS, a possible mechanistic link. Cell 101, 533–542.

Moussian, B., Schoof, H., Haecker, A., Jurgens, G., Laux, T., 1998. Role of the ZWILLE gene in the regulation of central shoot meristem cell fate during Arabidopsis embryogenesis. EMBO J. 17, 1799–1809.

Muangsan, N., Beclin, C., Vaucheret, H., Robertson, D., 2004. Geminivirus VIGS of endogenous genes requires SGS2/SDE1 and SGS3 and defines a new branch in the genetic pathway for silencing in plants. Plant J. 38, 1004–1014. https://doi.org/10.1111/j.1365-313X.2004.02103.x

Nguyen, H.D., Tomitaka, Y., Ho, S.Y.W., Duchêne, S., Vetten, H.-J., Lesemann, D., Walsh, J.A., Gibbs, A.J., Ohshima, K., 2013. Turnip Mosaic Potyvirus Probably First Spread to Eurasian Brassica Crops from Wild Orchids about 1000 Years Ago. PLoS One 8, e55336. https://doi.org/10.1371/journal.pone.0055336

Noah Fahlgren, T.A.M., Miya D. Howell,Edwards Allen, Sarah K. Dvorak, Amanda L. Alexander, and J.C.C., 2006. Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA Affects Developmental Timing and Patterning in Arabidopsis. Curr. Biol. 16, 939–944. https://doi.org/10.1016/j.cub.2006.03.065

Nonomura, K.-I., Morohoshi, A., Nakano, M., Eiguchi, M., Miyao, A., Hirochika, H., Kurata, N., 2007. A Germ Cell–Specific Gene of the ARGONAUTE Family Is Essential for the Progression of Premeiotic Mitosis and Meiosis during Sporogenesis in Rice. Plant Cell 19, 2583–2594. https://doi.org/10.1105/tpc.107.053199

Nuthikattu, S., McCue, A.D., Panda, K., Fultz, D., DeFraia, C., Thomas, E.N., Slotkin, R.K., 2013. The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21-22 nucleotide small interfering RNAs. Plant Physiol. 162, 116–31. https://doi.org/10.1104/pp.113.216481

Oa, W., Garcia-Ruiz, H., Takeda, A., Chapman, E.J., Sullivan, C.M., Fahlgren, N., Brempelis, K.J., Carrington, J.C., 2010. Arabidopsis RNA-Dependent RNA Polymerases and Dicer-Like Proteins in Antiviral Defense and Small Interfering RNA Biogenesis during Turnip Mosaic Virus Infection. Plant Cell 22, 481–496. https://doi.org/10.1105/tpc.109.073056

Odokonyero, D., Mendoza, M.R., Alvarado, V.Y., Zhang, J., Wang, X., Scholthof, H.B., 2015. Transgenic down-regulation of ARGONAUTE2 expression in Nicotiana benthamiana interferes with several layers of antiviral defenses. Virology 486, 209–218. https://doi.org/10.1016/j.virol.2015.09.008

Odokonyero, D., Mendoza, M.R., Moffett, P., Scholthof, H.B., 2017. *Tobacco rattle virus* (TRV)-Mediated Silencing of *Nicotiana benthamiana ARGONAUTES* (*NbAGOs*) Reveals New Antiviral Candidates and Dominant Effects of TRV- *NbAGO1*. Phytopathology PHYTO-02-17-004. https://doi.org/10.1094/PHYTO-02-17-0049-R

Okano, Y., Senshu, H., Hashimoto, M., Neriya, Y., Netsu, O., Minato, N., Yoshida, T., Maejima, K., Oshima, K., Komatsu, K., Yamaji, Y., Namba, S., 2014. In Planta Recognition of a Double-Stranded RNA Synthesis Protein Complex by a Potexviral RNA Silencing Suppressor. Plant Cell 26, 2168–2183. https://doi.org/10.1105/tpc.113.120535

Olmedo-Monfil, V., Durán-Figueroa, N., Arteaga-Vázquez, M., Demesa-Arévalo, E., Autran, D., Grimanelli, D., Slotkin, R.K., Martienssen, R.A., Vielle-Calzada, J.-P., 2010. Control of female gamete formation by a small RNA pathway in Arabidopsis. Nature 464, 628–632. https://doi.org/10.1038/nature08828

Olspert, A., Chung, B.Y., Atkins, J.F., Carr, J.P., Firth, A.E., 2015. Transcriptional slippage in the positive-sense RNA virus family Potyviridae. EMBO Rep. 16, 995–1004. https://doi.org/10.15252/embr.201540509

Pagán, I., Fraile, A., Fernandez-Fueyo, E., Montes, N., Alonso-Blanco, C., García-Arenal, F., 2010. Arabidopsis thaliana as a model for the study of plant-virus co-evolution. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 365, 1983–95. https://doi.org/10.1098/rstb.2010.0062

Palukaitis, P., Roossinck, M.J., Dietzgen, R.G., Francki, R.I.B., 1992. Cucumber MOSAIC Virus. Adv. Virus Res. 41, 281–348. https://doi.org/10.1016/S0065-3527(08)60039-1

Parent, J.-S., Bouteiller, N., Elmayan, T., Vaucheret, H., 2015. Respective contributions of Arabidopsis DCL2 and DCL4 to RNA silencing. Plant J. 81, 223–232. https://doi.org/10.1111/tpj.12720

Paudel, D.B., Ghoshal, B., Jossey, S., Ludman, M., Fatyol, K., Sanfaçon, H., 2018. Expression and antiviral function of ARGONAUTE 2 in Nicotiana benthamiana plants infected with two isolates of tomato ringspot virus with varying degrees of virulence. Virology 524, 127–139. https://doi.org/10.1016/J.VIROL.2018.08.016

Paulo, J., Machado, B., Calil, I.P., Santos, A.A., Fontes, E.P.B., 2017. Translational control in plant antiviral immunity. Genet. Mol. Biol. 40, 292–304. https://doi.org/10.1590/1678-4685-GMB-2016-0092

Pieterse, C.M.J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., Van Wees, S.C.M., 2012. Hormonal Modulation of Plant Immunity. Annu. Rev. Cell Dev. Biol. 28, 489–521.

https://doi.org/10.1146/annurev-cellbio-092910-154055

Pita, J.S., Morris, V., Roossinck, M.J., 2015. Mutation and Recombination Frequencies Reveal a Biological Contrast within Strains of Cucumber Mosaic Virus Downloaded from. J Virol 89, 6817–6823. https://doi.org/10.1128/JVI.01891-12

Pogany, J., Nagy, P.D., 2015. Activation of *Tomato Bushy Stunt Virus* RNA-Dependent RNA Polymerase by Cellular Heat Shock Protein 70 Is Enhanced by Phospholipids *In Vitro*. J. Virol. 89, 5714–5723. https://doi.org/10.1128/JVI.03711-14

Pogany, J., Nagy, P.D., 2012. p33-Independent Activation of a Truncated p92 RNA-Dependent RNA Polymerase of Tomato Bushy Stunt Virus in Yeast Cell-Free Extract. J. Virol. 86, 12025–12038.

Pontier, D., Picart, C., Roudier, F.O., Garcia, D., Lahmy, S., Azevedo, J., Alart, E., Le Laudié, M., Karlowski, W.M., Cooke, R., Colot, V., Voinnet, O., Lagrange, T., 2012. Molecular Cell Article NERD, a Plant-Specific GW Protein, Defines an Additional RNAi-Dependent Chromatin-Based Pathway in Arabidopsis. https://doi.org/10.1016/j.molcel.2012.07.027

Poulsen, C., Vaucheret, H., Brodersen, P., 2013. Lessons on RNA silencing mechanisms in plants from eukaryotic argonaute structures. Plant Cell 25, 22–37. https://doi.org/10.1105/tpc.112.105643

Powers, J.G., Sit, T.L., Qu, F., Morris, T.J., Kim, K.-H., Lommel, S.A., 2008. A Versatile Assay for the Identification of RNA Silencing Suppressors Based on Complementation of Viral Movement 21, 879–890.

Pradhan, M., Pandey, P., Gase, K., Sharaff, M., Singh, R., Sethi, A., Baldwin, I.T., Pandey, S.P., 2017. AGO8 mediates defense response against herbivores 2. Plant Physiol. https://doi.org/10.1104/pp.17.00702

Pumplin, N., Voinnet, O., 2013. RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence. Nat. Rev. Microbiol. 11, 745–60.

Qi, X., Bao, F.S., Xie, Z., 2009. Small RNA deep sequencing reveals role for Arabidopsis thaliana RNA-dependent RNA polymerases in viral siRNA biogenesis. PLoS One 4, e4971. https://doi.org/10.1371/journal.pone.0004971

Qi, Y., Denli, A.M., Hannon, G.J., 2005. Technique Biochemical Specialization within Arabidopsis RNA Silencing Pathways. Mol. Cell 19, 421–428. https://doi.org/10.1016/j.molcel.2005.06.014

Qu, F., Morris, T.J., 2005. Suppressors of RNA silencing encoded by plant viruses and their role in viral infections. FEBS Lett.

Qu, F., Ye, X., Morris, T.J., 2008. Arabidopsis DRB4, AGO1, AGO7, and RDR6 participate in a DCL4-initiated antiviral RNA silencing pathway negatively regulated by DCL1. Proc. Natl. Acad. Sci. U. S. A. 105, 14732–7. https://doi.org/10.1073/pnas.0805760105

Raja, Priya, Jackel, J.N., Li, S., Heard, I.M., Bisaro, D.M., 2014. Arabidopsis double-stranded RNA binding protein DRB3 participates in methylation-mediated defense against geminiviruses. J. Virol. 88, 2611–22. https://doi.org/10.1128/JVI.02305-13

Raja, P., Jackel, J.N., Li, S., Heard, I.M., Bisaro, D.M., 2014. Arabidopsis Double-Stranded RNA Binding Protein DRB3 Participates in Methylation-Mediated Defense against Geminiviruses. J. Virol. 88, 2611–2622. https://doi.org/10.1128/JVI.02305-13

Raja, P., Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008a. Viral Genome Methylation as an Epigenetic Defense against Geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, Priya, Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008. Viral genome methylation as an epigenetic defense against geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, P., Sanville, B.C., Buchmann, R.C., Bisaro, D.M., 2008b. Viral Genome Methylation as an Epigenetic Defense against Geminiviruses. J. Virol. 82, 8997–9007. https://doi.org/10.1128/JVI.00719-08

Raja, P., Wolf, J.N., Bisaro, D.M., 2010. RNA silencing directed against geminiviruses: Post-transcriptional and epigenetic components. Biochim. Biophys. Acta - Gene Regul. Mech. 1799, 337–351. https://doi.org/10.1016/j.bbagrm.2010.01.004

Reinhart, B.J., Weinstein, E.G., Rhoades, M.W., Bartel, B., Bartel, D.P., 2002. MicroRNAs in plants. Genes Dev.

Revers, F., García, J.A., 2015. Molecular biology of potyviruses, in: Advances in Virus Research. Academic Press Inc., pp. 101–199. https://doi.org/10.1016/bs.aivir.2014.11.006

Reyero-Saavedra, M. del R., Qiao, Z., Sánchez-Correa, M. del S., Reyes, J.L., Covarrubias, A.A., Libault, M., Valdés-López, O., 2017. Gene Silencing of Argonaute5 Negatively Affects the Establishment of the Legume-Rhizobia Symbiosis. Genes (Basel). 5, 1–19. https://doi.org/10.3390/genes8120352

Rivas, F. V, Tolia, N.H., Song, J.-J., Aragon, J.P., Liu, J., Hannon, G.J., Joshua-Tor, L., 2005. Purified Argonaute2 and an siRNA form recombinant human RISC. Nat. Struct. Mol. Biol. 12, 340–349. https://doi.org/10.1038/nsmb918

Rodríguez-Leal, D., Castillo-Cobián, A., Rodríguez-Arévalo, I., Vielle-Calzada, J.-P., 2016. A Primary Sequence Analysis of the ARGONAUTE Protein Family in Plants. Front. Plant Sci. 7, 1347. https://doi.org/10.3389/fpls.2016.01347

Roossinck, M.J., 2002. Evolutionary history of Cucumber mosaic virus deduced by phylogenetic analyses. J. Virol. 76, 3382–7. https://doi.org/10.1128/jvi.76.7.3382-3387.2002

Roossinck, M.J., 2001. Cucumber mosaic virus, a model for RNA virus evolution. Mol. Plant Pathol. 2, 59–63. https://doi.org/10.1046/j.1364-3703.2001.00058.x

Roth, B.M., Pruss, G.J., Vance, V.B., 2004. Review-Plant viral suppressors of RNA silencing. Virus Res. 102, 97–108. https://doi.org/10.1016/j.virusres.2004.01.020

Schepetilnikov, M. V., Manske, U., Solovyev, A.G., Jr, A.A.Z., Schiemann, J., Morozov, S.Y., 2005. The hydrophobic segment of Potato virus X TGBp3 is a major determinant of the protein intracellular trafficking. J. Gen. Virol. 86, 2379–2391. https://doi.org/10.1099/vir.0.80865-0

Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Schölkopf, B., Weigel, D., Lohmann, J.U., 2005. A gene expression map of Arabidopsis thaliana development. Nat. Genet. 37, 501–506. https://doi.org/10.1038/ng1543

Scholthof, H.B., Alvarado, V.Y., Vega-Arreguin, J.C., Ciomperlik, J., Odokonyero, D., Brosseau, C., Jaubert, M., Zamora, A., Moffett, P., 2011. Identification of an ARGONAUTE for antiviral RNA silencing in Nicotiana benthamiana. Plant Physiol. 156,

1548–55. https://doi.org/10.1104/pp.111.178764

Schott, G., Mari-Ordonez, A., Himber, C., Alioua, A., Voinnet, O., Dunoyer, P., 2012. Differential effects of viral silencing suppressors on siRNA and miRNA loading support the existence of two distinct cellular pools of ARGONAUTE1. EMBO J. 31, 2553–65. https://doi.org/10.1038/emboj.2012.92

Schuck, J., Gursinsky, T., Pantaleo, V., Burgy??n, J., Behrens, S.E., 2013a. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic Acids Res. 41, 5090–5103.

Schuck, J., Gursinsky, T., Pantaleo, V., Burgyán, J., Behrens, S.-E., 2013b. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic Acids Res. 41, 5090–103. https://doi.org/10.1093/nar/gkt193

Senshu, H., Ozeki, J., Komatsu, K., Hashimoto, M., Hatada, K., Aoyama, M., Kagiwada, S., Yamaji, Y., Namba, S., 2017. Variability in the level of RNA silencing suppression caused by triple gene block protein 1 (TGBp1) from various potexviruses during infection. https://doi.org/10.1099/vir.0.008243-0

Senshu, H., Ozeki, J., Komatsu, K., Hashimoto, M., Hatada, K., Aoyama, M., Kagiwada, S., Yamaji, Y., Namba, S., 2009. Variability in the level of RNA silencing suppression caused by triple gene block protein 1 (TGBp1) from various potexviruses during infection. J. Gen. Virol. https://doi.org/10.1099/vir.0.008243-0

Shamandi, N., Zytnicki, M., Charbonnel, C., Elvira-Matelot, E., Bochnakian, A., Comella, P., Mallory, A.C., Lepère, G., Sáez-Vásquez, J., Vaucheret, H., 2015a. Plants Encode a General siRNA Suppressor That Is Induced and Suppressed by Viruses. PLoS Biol. 13.

Shamandi, N., Zytnicki, M., Charbonnel, C., Elvira-Matelot, E., Bochnakian, A., Comella, P., Mallory, A.C., Lepère, G., Sáez-Vásquez, J., Vaucheret, H., 2015b. Plants Encode a General siRNA Suppressor That Is Induced and Suppressed by Viruses. PLOS Biol. 13, e1002326. https://doi.org/10.1371/journal.pbio.1002326

Silhavy, D., Molnár, A., Lucioli, A., Szittya, G., Hornyik, C., Tavazza, M., Burgyán, J., 2002. A viral protein suppresses RNA silencing and binds silencing-generated, 21- to 25-nucleotide

double-stranded RNAs. EMBO J. 21, 3070–80. https://doi.org/10.1093/emboj/cdf312

Solovyev, A.G., Kalinina, N.O., Morozov, S.Y., 2012. Recent Advances in Research of Plant Virus Movement Mediated by Triple Gene Block. Front. Plant Sci. https://doi.org/10.3389/fpls.2012.00276

Solovyev, A.G., Stroganova, T.A., Zamyatnin, A.A., Fedorkin, O.N., Schiemann, J., Morozov, S.Y., 2000. Subcellular Sorting of Small Membrane-Associated Triple Gene Block Proteins: TGBp3-Assisted Targeting of TGBp2. Virology 269, 113–127. https://doi.org/10.1006/VIRO.2000.0200

Song, J.-J., Smith, S.K., HannonL, G.J., Joshua-Tor, L., 2004. Crystal Structure of Argonaute and Its Implications for RISC Slicer Activity. Science (80-.). 305, 1434–1437. https://doi.org/10.1126/science.1102514

Song, M.-S., Rossi, J.J., 2017. Molecular mechanisms of Dicer: endonuclease and enzymatic activity. Biochem. J. 474, 1603–1618. https://doi.org/10.1042/bcj20160759

Stael, S., Kmiecik, P., Willems, P., Van Der Kelen, K., Coll, N.S., Teige, M., Van Breusegem, F., 2015. Plant innate immunity--sunny side up? Trends Plant Sci. 20, 3–11. https://doi.org/10.1016/j.tplants.2014.10.002

Syller, J., 2012. Facilitative and antagonistic interactions between plant viruses in mixed infections. Mol. Plant Pathol. https://doi.org/10.1111/j.1364-3703.2011.00734.x

Szittya, G., Moxon, S., Pantaleo, V., Toth, G., Rusholme Pilcher, R.L., Moulton, V., Burgyan, J., Dalmay, T., 2010. Structural and functional analysis of viral siRNAs. PLoS Pathog. 6, e1000838. https://doi.org/10.1371/journal.ppat.1000838

Takeda, A., Iwasaki, S., Watanabe, T., Utsumi, M., Watanabe, Y., 2008. The Mechanism Selecting the Guide Strand from Small RNA Duplexes is Different Among Argonaute Proteins. Plant Cell Physiol. 49, 493–500. https://doi.org/10.1093/pcp/pcn043

Tsuzuki, M., Takeda, A., Watanabe, Y., 2014. Recovery of dicer-like 1 -late flowering phenotype by miR172 expressed by the noncanonical DCL4-dependent biogenesis pathway. RNA 20, 1320–1327. https://doi.org/10.1261/rna.044966.114

Tucker, M.R., Okada, T., Hu, Y., Scholefield, a., Taylor, J.M., Koltunow, a. M.G., 2012. Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in Arabidopsis. Development 139, 1399–1404. https://doi.org/10.1242/dev.075390

Vance, V., 2001. RNA Silencing in Plants--Defense and Counterdefense. Science (80-.). 292, 2277–2280. https://doi.org/10.1126/science.1061334

Várallyay, É., Oláh, E., Havelda, Z., 2014. Independent parallel functions of p19 plant viral suppressor of RNA silencing required for effective suppressor activity. Nucleic Acids Res. 42, 599–608. https://doi.org/10.1093/nar/gkt846

Vaucheret, H., 2008. Plant ARGONAUTES. Trends Plant Sci.

Vaucheret, H., Mallory, A.C., Bartel, D.P., 2006. AGO1 Homeostasis Entails Coexpression of MIR168 and AGO1 and Preferential Stabilization of miR168 by AGO1. Mol. Cell 22, 129–136.

Vaucheret, H., Vazquez, F., Crété, P., Bartel, D.P., 2004. The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. Genes Dev. 18, 1187–1197. https://doi.org/10.1101/gad.1201404

Verchot-Lubicz, J., Torrance, L., Solovyev, A.G., Morozov, S.Y., Jackson, A.O., Gilmer, D., 2010. Varied Movement Strategies Employed by Triple Gene Block–Encoding Viruses 23, 1231–1247. https://doi.org/10.1094

Verlaan, M.G., Hutton, S.F., Ibrahem, R.M., Kormelink, R., Visser, R.G.F., Scott, J.W., Edwards, J.D., Bai, Y., 2013. The Tomato Yellow Leaf Curl Virus Resistance Genes Ty-1 and Ty-3 Are Allelic and Code for DFDGD-Class RNA-Dependent RNA Polymerases. PLoS Genet. 9.

Vickers, T.A., Koo, S., Bennett, C.F., Crooke, S.T., Dean, N.M., Baker, B.F., 2003. Efficient reduction of target RNAs by small interfering RNA and RNase H-dependent antisense agents. A comparative analysis. J. Biol. Chem. 278, 7108–7118.

Voinnet, O., Lederer, C., Baulcombe, D.C., 2000. A Viral Movement Protein Prevents Spread of the Gene Silencing Signal in Nicotiana benthamiana. Cell 103, 157–167.

https://doi.org/10.1016/S0092-8674(00)00095-7

Wang, J., Mei, J., Ren, G., 2019. Plant microRNAs: Biogenesis, Homeostasis, and Degradation. Front. Plant Sci. 10, 360. https://doi.org/10.3389/fpls.2019.00360

Wang, M.-B., Metzlaff, M., 2005. RNA silencing and antiviral defense in plants. Curr. Opin. Plant Biol. 8, 216–222. https://doi.org/10.1016/j.pbi.2005.01.006

Wang, X.-B., Jovel, J., Udomporn, P., Wang, Y., Wu, Q., Li, W.-X., Gasciolli, V., Vaucheret, H., Ding, S.-W., 2011. The 21-Nucleotide, but Not 22-Nucleotide, Viral Secondary Small Interfering RNAs Direct Potent Antiviral Defense by Two Cooperative Argonautes in Arabidopsis thaliana. Plant Cell 23, 1625–1638. https://doi.org/10.1105/tpc.110.082305

Wang, X.-B., Wu, Q., Ito, T., Cillo, F., Li, W.-X., Chen, X., Yu, J.-L., Ding, S.-W., 2010. RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 107, 484–489. https://doi.org/10.1073/pnas.0904086107

Wassenegger, M., Krczal, G., 2006. Nomenclature and functions of RNA-directed RNA polymerases. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2006.01.003

Wei, T., Wang, L., Zhou, X., Ren, X., Dai, X., Liu, H., 2015. PopW activates PAMP-triggered immunity in controlling tomato bacterial spot disease. Biochem. Biophys. Res. Commun. 463, 746–750.

Wei, W., Ba, Z., Gao, M., Wu, Y., Ma, Y., Amiard, S., White, C.I., Rendtlew Danielsen, J.M., Yang, Y.-G., Qi, Y., 2012. A Role for Small RNAs in DNA Double-Strand Break Repair. Cell 149, 101–112. https://doi.org/10.1016/j.cell.2012.03.002

Willmann, M.R., Endres, M.W., Cook, R.T., Gregory, B.D., 2011. The Functions of RNA-Dependent RNA Polymerases in Arabidopsis. Arab. B. 9, e0146. https://doi.org/10.1199/tab.0146

Wu, J., Yang, Z., Wang, Y., Zheng, L., Ye, R., Ji, Y., Zhao, S., Ji, S., Liu, R., Xu, L., Zheng, H., Zhou, Y., Zhang, X., Cao, X., Xie, L., Wu, Z., Qi, Y., Li, Y., 2015. Viral-inducible Argonaute18 confers broad-spectrum virus resistance in rice by sequestering a host microRNA. Elife 4. https://doi.org/10.7554/eLife.05733

Xie, Z., Johansen, L.K., Gustafson, A.M., Kasschau, K.D., Lellis, A.D., Zilberman, D., Jacobsen, S.E., Carrington, J.C., 2004. Genetic and functional diversification of small RNA pathways in plants. PLoS Biol. 2, E104. https://doi.org/10.1371/journal.pbio.0020104

Xu, D., Zhou, G., 2017. Characteristics of siRNAs derived from Southern rice black-streaked dwarf virus in infected rice and their potential role in host gene regulation. Virol. J. 14, 27. https://doi.org/10.1186/s12985-017-0699-3

Ye, R., Wang, W., Iki, T., Liu, C., Wu, Y., Ishikawa, M., Zhou, X., Qi, Y., 2012. Cytoplasmic Assembly and Selective Nuclear Import of Arabidopsis ARGONAUTE4/siRNA Complexes. Mol. Cell 46, 859–870. https://doi.org/10.1016/j.molcel.2012.04.013

Yoshikawa, M., 2013. Biogenesis of trans-acting siRNAs, endogenous secondary siRNAs in plants. Genes Genet. Syst. 88, 77–84.

Yoshikawa, M., Iki, T., Tsutsui, Y., Miyashita, K., Poethig, R.S., Habu, Y., Ishikawa, M., 2013. 3′ fragment of miR173-programmed RISC-cleaved RNA is protected from degradation in a complex with RISC and SGS3. PNAS 110, 4117–4122. https://doi.org/10.1073/pnas.1217050110

Yu, X.D., Liu, Z.C., Huang, S.L., Chen, Z.Q., Sun, Y.W., Duan, P.F., Ma, Y.Z., Xia, L.Q., 2016. RNAi-mediated plant protection against aphids. Pest Manag. Sci.

Zhang, H., Xia, R., Walbot, V., 2015. Evolution, functions, and mysteries of plant ARGONAUTE proteins. Curr. Opin. Plant Biol. 27, 84–90. https://doi.org/10.1016/J.PBI.2015.06.011

Zhang, Xiaoming, Xia, J., Lii, Y., Barrera-Figueroa, B., Zhou, X., Gao, S., Lu, L., Niu, D., Liang, W., Chen, Z., Leung, C., Wong, T., Guo, J., Li, Y., Liu, R., Zhu, J.-K., Zhang, W., Jin, H., Zhang, H., 2012. Genome-wide analysis of plant nat-siRNAs reveals insights into their distribution, biogenesis and function. Genome Biol. 13, R20.

Zhang, X., Yuan, Y.-R., Pei, Y., Lin, S.-S., Tuschl, T., Patel, D.J., Chua, N.-H., 2006. Cucumber mosaic virus-encoded 2b suppressor inhibits Arabidopsis Argonaute1 cleavage activity to counter plant defense. Genes Dev. 20, 3255–68. https://doi.org/10.1101/gad.1495506

Zhang, X., Zhang, X., Singh, J., Li, D., Qu, F., 2012. Temperature-Dependent Survival of Turnip

Crinkle Virus-Infected Arabidopsis Plants Relies on an RNA Silencing-Based Defense That Requires DCL2, AGO2, and HEN1. J. Virol. 86, 6847–6854. https://doi.org/10.1128/JVI.00497-12

Zhang, X., Zhao, H., Gao, S., Wang, W.-C., Katiyar-Agarwal, S., Huang, H.-D., Raikhel, N., Jin, H., 2011. Arabidopsis Argonaute 2 Regulates Innate Immunity via miRNA393∗-Mediated Silencing of a Golgi-Localized SNARE Gene, MEMB12. Mol. Cell 42, 356–366. https://doi.org/10.1016/j.molcel.2011.04.010

Zhang, Xiuchun, Zhang, Xiaofeng, Singh, J., Li, D., Qu, F., 2012. Temperature-dependent survival of Turnip crinkle virus-infected arabidopsis plants relies on an RNA silencing-based defense that requires dcl2, AGO2, and HEN1. J. Virol. 86, 6847–54. https://doi.org/10.1128/JVI.00497-12

Zhang, Z., Liu, X., Guo, X., Wang, X.-J., Zhang, X., 2016. Arabidopsis AGO3 predominantly recruits 24-nt small RNAs to regulate epigenetic silencing. Nat. Plants 2, 16049. https://doi.org/10.1038/nplants.2016.49

Zheng, X., Fahlgren, N., Abbasi, A., Berry, J.C., Carrington, J.C., 2019. Antiviral ARGONAUTEs Against Turnip Crinkle Virus Revealed by Image-Based Trait Analysis. Plant Physiol. 180, 1418–1435. https://doi.org/10.1104/pp.19.00121

Zheng, X., Zhu, J., Kapoor, A., Zhu, J.-K., Liu, J., Chen, X., Harmer, S., Zilberman, D., Pikaard, C., Watson, J., Rezaian, A., Dennis, E., Waterhouse, P., Martienssen, R., 2007. Role of Arabidopsis AGO6 in siRNA accumulation, DNA methylation and transcriptional gene silencing. EMBO J. 26, 1691–701. https://doi.org/10.1038/sj.emboj.7601603

Zhou, Y., Honda, M., Duan, L., Zhang Correspondence, X., Zhu, H., Zhang, Z., Guo, X., Li, T., Li, Z., Peng, X., Nakajima, K., Zhang, X., 2015. Spatiotemporal Sequestration of miR165/166 by Arabidopsis Argonaute10 Promotes Shoot Apical Meristem Maintenance. CellReports 10, 1819–1827. https://doi.org/10.1016/j.celrep.2015.02.047

Zhu, H., Hu, F., Wang, R., Zhou, X., Sze, S.-H., Liou, L.W., Barefoot, A., Dickman, M., Zhang, X., Qi, Y, Chen, S., Hannon, G., Qi, Y, Chen, X., Chen, X., Wu, Z., Qi, Yijun, Li, Y., 2011. Arabidopsis Argonaute10 Specifically Sequesters miR166/165 to Regulate Shoot Apical

Meristem Development. Cell 145, 242–256. https://doi.org/10.1016/j.cell.2011.03.024

Zilberman, D., Cao, X., Jacobsen, S.E., 2003. ARGONAUTE4 control of locus-specific siRNA accumulation and DNA and histone methylation. Science 299, 716–9. https://doi.org/10.1126/science.1079695

Zust, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., Barchet, W., Diamond, M.S., Siddell, S.G., Ludewig, B., Thiel, V., 2011. Ribose 2'-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. Nat Immunol 12, 137–143.